ZC3HAV1

PDB:2X5Y

Revision

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Entry Clone Accession:BX571742
Entry Clone Source:DKFZ
SGC Clone Accession:ZC3HAV1A-s002
Tag:N-terminal hexahistidine tag: mhhhhhhssgvdlgtenlyfqsm
Host:*Escherichia coli* BL21(DE3) R3 pRARE

Construct

Prelude:
Sequence:

```
mhhhhhhssgvdlgtenlyfqsm*KKYKLSEIHHLHPEYVRVSEHFKASMKNFKIEKIKKIENSELLDKFTWKKSQMKEEGKLLFYA
TSRAYVESICSNNFDSFLHETHENKYGKGIYFAKDAIYSHKNCPYDAKNVVMFVAQVLVGKFIEGNITYTSPPPQFDSCVDTRSNPS
VFVIFQKDQVYPQYVIEYTED
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Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Fresh overnight cultures of *E. coli* strain BL21(DE3) R3 pRARE cells (including 100 µg/ml kanamycin and 34 µg/ml chloramphenicol) transformed with ZC3HAV1 expression construct were used to inoculate 4.5 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and anti-foam 204 in three 2-liter flasks. Cells were grown in a large scale expression system (Harbinger Biotechnology and Engineering) at 37°C until the OD600 reached ~2. The culture was down-tempered to 18°C for 1 h. Expression of ZC3HAV1 was induced by adding 0.5 mM IPTG and growth continued over night at 18°C. Cells were harvested by centrifugation at 4400 x *g* for 10 min. The pellet (100g wet cell weight) was resuspended in 150 ml lysis buffer supplemented with Complete EDTA-free Protease Inhibitor (Roche Biosciences) and benzonase Suspended cells were stored at -80°C until further use.

Purification

Procedure
Columns
IMAC: Ni-charged 5 ml HiTrap Chelating HP (GE Healthcare)

Gel filtration column: HiLoad 16/60 Superdex 75 Prep Grade (GE Healthcare)

Purification
Purification of the protein was performed on an ÄKTAxpress system (GE Healthcare). Prior to purification, IMAC column was equilibrated with IMAC wash1 buffer and gel filtration column with gel filtration buffer. The filtered lysate was loaded onto the IMAC column, and therafter washed with IMAC wash1 buffer followed by IMAC wash2 buffer. Bound protein was eluted from the IMAC column with IMAC elution buffer and automatically loaded onto the gel filtration column. Eluting fractions were analyzed by SDS-PAGE and target protein was pooled. Fresh TCEP was added to a final concentration of 2 mM. Purified protein was concentrated using an Amicon Ultra-15 centrifugal filter device (Millipore, 10,000 MWCO) to 48 mg/ml.

His-tag removal
The N-terminal histidine tag was proteolytically removed by incubating the target protein with His-tagged TEV protease at a molar ratio of 50:1 in 4 ºC overnight. The proteolytic reaction went to completion, as judged by SDS-PAGE. The target protein was subsequently purified from tag and protease by passing it over a Ni-charged HiTrap chelating column (GE Healthcare) pre-equilibrated with GF buffer and using the Äkta Express. The cleaved protein was eluted with IMAC wash2 buffer. The protein was concentrated and the buffer changed to 20mM Tris-HCl, 150mM NaCl using an Amicon Ultra-15 centrifugal filter device, 10,000 NMWL (Millipore). The concentration was measured to 40.6 mg/ml. The identity of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The cell suspension was quickly thawed in warm water. Cells were disrupted by sonication (Vibra-Cell, Sonics) at 80% amplitude for 3 min effective time (pulsed 4s on, 4s off) and cell debris was removed by centrifugation (49000 x *g*, 20 min, 4 ºC). The supernatant was decanted and filtered through a 0.45 µm flask filter.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained by the sitting drop vapour diffusion method in a 96-well plate. 0.2 µl protein solution (40.6 mg/ml) was mixed with 0.4 µl of well solution consisting of 20% PEG3350, 0.2M sodium fluoride. The plate was incubated at 4 ºC. Crystals grew for 10 days wherafter they were quickly transferred to a cryo solution consisting of 22% PEG3350, 0.2M sodium fluoride, 0.02M Tris-HCl pH 7.5, 0.8mM NAD and 20% Glycerol and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data to 1.05 Å resolution was collected at BESSY beamline BL14-1.
Data Processing: Data were indexed and integrated in space group $P2_12_12_1$ using XDS software. The structure was solved by molecular replacement using the structure of the catalytic domain of the previously determined human PARP12 (pdb: 2pqf) as model template. Molrep was used to solve the structure. The asymmetric unit contained one protein monomer. The cell dimensions are a = 47.9Å, b = 54.1Å, c = 69.3Å. Arp/wARP was used for initial automatic model building, Refmac5 was used for refinement and Coot for manual model building. Data in the interval 35.0-1.05 Å resolution were used and refined to R = 13.05% and R_{free} = 14.93%. Coordinates for the crystal structure were deposited in the Protein Data Bank, with accession code 2x5y.