PARP15 (macro domain)

PDB:3KH6

Revision

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Entry Clone Accession:BC101701
Entry Clone Source:MGC, Open Biosystems
SGC Clone Accession:PARP15A-s002
Tag:N-terminal hexahistidine tag: MHHHHHHSSGVDLGTENLYFQSM
Host:BL21(DE3) R3 pRARE

Construct

Prelude:
Sequence:

```
MHHHHHHSSGVDLGTENLYFQSMTAYEMKIGAITFQVATGDIATEQVDVIVNSTARTFNRKSGVSRAILEGAGQAVESECAVLAAQP
HRDFIITPGGCLKCKIIIHVPGGKDVRKTVTSVLEECEQRKYTSVSLPAIGTGNAGKNPITVADNIIDAIVDFSSQHSTPSLKTVKV
VIFQPELLNIFYDSMKKRDLSASLN
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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 PARP15 expression construct were used to inoculate 1.5 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and anti-foam 204 in a 2-liter flask. 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 PARP15 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 (36g wet cell weight) was resuspended in 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 1 ml HiTrap Chelating HP (GE Healthcare)
Gel filtration column: HiLoad 16/60 Superdex 75 Prep Grade (GE Healthcare)

Procedure
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. The protein was concentrated using an Amicon Ultra-15 centrifugal filter device, 10,000 MWCO (Millipore) to 39.0 mg/ml in a volume of 0.6 ml. The expected mass of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The cell suspension was quickly thawed in 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. 0.1 µl protein solution (38 mg/ml) including 5 mM ADP-ribose was mixed with 0.2 µl of well solution consisting of 20% PEG6000, 0.1M Na-Acetate 5.0, 0.2M NaCl. The plate was incubated at 4 ºC. Crystals grew in 28 days wherafter they were quickly transferred to a cryo solution consisting of 22% PEG6000, 0.1M Na-Acetate 5.0, 0.2M NaCl, 20% Glycerol and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data to 2.2 Å resolution was collected at BESSY beamline BL14-2.
Data Processing: Data were indexed and integrated in space group C2221 using XDS software. The structure was solved by molecular replacement using the structure of putative phosphatase of E. coli (pdb: 1SPV) as model template. Balbes was used to solve the structure. The asymmetric unit contained one protein monomer. The cell dimensions a = 68.10Å, b = 91.19Å, c = 62.85Å.

Refmac5 was used for refinement and Coot for manual model building. Data in the interval 35.0-2.2 Å resolution were used and refined to R = 18.16% and Rfree = 23.19%. Coordinates for the crystal structure were deposited in the Protein Data Bank, with accession code 3KH6.