PARP14

PDB:3Q71

Revision

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Tag:N-terminal hexahistidine tag: mhhhhhhssgvdlgtenlyfq*sm
Host:*Escherichia coli* BL21(DE3) R3 pRARE

Construct

Prelude:
Sequence:

MHHHHHHSSGVDLGTENLYFQSMGKTSWEKGSLVSPGGLQMLLVKEGVQNAKTDVVVNSVPLDLVLSRGPLSKSLLEKAGPELQEEL
DTVGQGVAVSMGTVLKTSSWNLDCRYVLHVVAPEWRNGSTSSLKIMEDIIRECMEITESLSLKSIAFPAIGTGNLGFPKNIFAELII
SEVFKFSSKNQLKTLQEVHFLLHPSDHENIQAFSDEFARRANGNLVS

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 PARP14 expression construct were used to inoculate 3 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and anti-foam in two 2-liter flasks. Cells were grown in a large scale expression system (Harbinger Biotechnology and Engineering) at 37°C until the OD600 reached ~1. The culture was down-tempered to 18°C for 1 h. Expression of TNKS2 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 (47g wet cell weight) was resuspended in 140ml 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 to 11.7 mg/ml. Mass spectrometry was used to verify the molecular mass.

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 of PARP14 were grown using vapor diffusion at 4°C by mixing 0.15ul of protein solution at 11.2 mg/ml including 4mM ADP-ribose, 4mM MgCl2 and 0.1ul reservoir solution containing 1.4M Ammonium Sulfate, 0.1M Sodium Acetate pH 5.1, 0.4M Sodium Chloride. Crystal was mounted 2 months after setup, size was (approx. 50 µm × 20 µm × 20 µm). The crystal was transferred to a cryo-protectant consisting of 1.4M Ammonium Sulfate, 0.1M Sodium Acetate pH 4.9, 0.2M Sodium Chloride, 28% Glycerol and frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data to 2.2 Å resolution was collected at MAX II beamline I911-5 using wavelength 0.90770Å.
Data Processing: Data were indexed and integrated in space group $P3_221$ using XDS software.

The structure was solved by molecular replacement using the macro domain of human histone macroH2A1.1 in complex with ADP-ribose (pdb: 3iid) as model template. Balbes was used to solve the structure. The asymmetric unit contained one protein monomer. The cell dimensions are a = b= 66.35Å, c = 110.69Å. Refmac5 was used for refinement and Coot for model building. Data in the interval 28.7-2.2 Å resolution were used and refined to R = 19.09% and Rfree = 22.28%. Coordinates for the crystal structure were deposited in the Protein Data Bank, with accession code 3Q71.