

PARP2

PDB:3KCZ

Revision

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Entry Clone Accession:E0050

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SGC Clone Accession:PARP2A-s002, PARP2A-s003

Tag:N-terminal hexahistidine tag: MHHHHHHSSGVDLG TENLYFQSM

Host:Escherichia coli BL21(DE3) R3 pRARE

Construct

Prelude:

Sequence:

MHHHHHHSSGVDLG TENLYFQSM DLRVQELIKLICNVQAMEEMMEMKYNTKKAPLGKLTVAQIKAGYQSLKKIEDCIRAGQHGRAL
MEACNEFYTRIPHD FGLRTPPLIRTQKELSEKIQLLEALGDIEIAIKLVKTELQSPEHPLDQHYRNLHCALRPLDHESYEFKVISQY
LQSTHAPTHSDYTMTLLDLFEVEKDGEKEAFREDLHNRMLLWHGSRMSNWVGILSHGLRIAHPEAPITGYMFGKGIYFADMSSKSAN
YCFASRLKNTGLLLLSEVALGQCNELLEANPKAEGLLQGHSTKGLGKMAPSSAHFVTLNGSTVPLGPASDTGILNPDGYTLNNEY
IVYNPNQVRMRYLLKVQFNF

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 PARP2 expression construct were used to inoculate 4.5 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and anti-foam PPG P2000 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 PARP2 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 (107g wet cell weight) was resuspended in 90ml 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 200 Prep Grade (GE Healthcare)

Procedure

Purification of the protein was performed on an ÄKTExpress 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 thereafter 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 37.2 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 °C). The supernatant was decanted and filtered through a 0.45 µm flask filter.

Concentration:

Ligand

3-aminobenzamide

Crystallization: Crystals were obtained by the sitting drop vapour diffusion method in a 96-well plate. 0.1 µl protein solution (30 mg/ml) including 10 mM 3-aminobenzamide was mixed with 0.2 µl of well solution consisting of 22% PEG3350, 0.1M Tris-HCl pH 9.0. The plate was incubated at 4 °C. Crystals grew in 5 days whereafter they were quickly transferred to a cryo solution consisting of 22% PEG3350, 0.1M Tris-HCl pH 9.0, 20% Glycerol, 0.2M NaCl, and flash frozen in liquid nitrogen.

NMR Spectroscopy:

Data Collection: Diffraction data to 2.0 Å resolution was collected at DIAMOND beamline I04.

Data Processing: Data were indexed and integrated in space group P21 using XDS software. The structure was solved by molecular replacement using the structure of the catalytic domain of mouse parp2 (pdb: 1gs0) as model template. Molrep was used to solve the structure. The asymmetric unit contained two protein monomers. The space group was P21 with cell dimensions $a = 58.28\text{Å}$, $b = 134.72\text{Å}$, $c = 58.25\text{Å}$, $\beta = 117.67^\circ$. Arp/warp was used for initial automatic model building, Refmac5 for refinement and Coot for manual model building. Data in the interval 41.0-2.0 Å resolution were used and refined to $R = 18.99\%$ and $R_{\text{free}} = 23.67\%$. Coordinates for the crystal structure were deposited in the Protein Data Bank, with accession code 3kcz.