PARP10

PDB:3HKV

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:AK027370, GI:157738665
Entry Clone Source: New Energy and Industrial Technology Development Organization, Japan (NEDO)
SGC Clone Accession:PARP10A-s003
Tag:C-terminal hexahistidine tag: ahhhhhh
Host:*Escherichia coli* BL21(DE3) R3 pRARE

Construct

Prelude:
Sequence:

AGQTLKGPWNNLERLAENTGEFQEVVRAFYDTLDAARSSIRVVRVERVSHPLLQQQYELYRERLLQRCERRPVEQVLYHGTTAPAVP
DICAHGFNRSFCGRNATVYGKGVYFAKRASLSVQDRYSPPNADGHKAVFVARVLTGDYGQGRRGLRAPPLRGPGHVLLRYDSAMDCI
CQPSIFVIFHDTQALPTHLITCEHVPRASPDDPSGahhhhhh

Vector:pNIC-CH

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 PARP10 expression construct were used to inoculate 0.75 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and anti-foam 204 in a 1 liter flask. Cells were grown in a large scale expression system (Harbinger Biotechnology and Engineering) at 37°C until the OD600 reached ~1.9. The culture was down-tempered to 18°C for 1 h. Expression of PARP10 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 (24.5 g wet cell weight) was resuspended in lysis buffer supplemented with Complete EDTA-free Protease Inhibitor (Roche Biosciences) and 4 ml benzonase (1000 U). 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 columns were equilibrated with IMAC wash1 buffer and gel filtration columns with gel filtration buffer. The filtered lysate was loaded onto IMAC columns, and columns were 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 and 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 NMWL (Millipore) to 14.2 mg/ml in a volume of 1.0 ml. Mass spectrometry showed -100 Da difference from the expected mass. Re-sequencing of the expression plasmid revealed absence of the initiator codon (Met1) as well as a mutation coding for Val979Met.

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 (49,000 x *g*, 20 min, 4 ºC). The supernatant was decanted and filtered through a 0.45 µm flask filter.
Concentration:
Ligand
3-aminobenzamide**MassSpec:**
Crystallization: Crystals were obtained by the sitting drop vapour diffusion method in a 96-well plate. 0.1 µl protein solution (14.1 mg/ml) including 10 mM 3-aminobenzamide was mixed with 0.1 µl of well solution consisting of 0.49 M NaH2PO4 and 0.9 M K2HPO4. The plate was incubated at 4 ºC and crystals appeared after five days and grew to size (approx. 90 µm × 60 µm × 30 µm). The crystals were quickly transferred to a cryo solution consisting of well solution complemented with 20% L-(+)-2,3-Butanediol, and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data to 2.1 Å resolution was collected at MAX-II beamline I911-3.
Data Processing: Data were indexed and integrated in space group P41212 with the XDS package. The structure was solved by molecular replacement using the structure of the catalytic domain of PARP-15 (PDB: 3blj) as model template. Chainsaw was used to edit the model and PHASER to solve the structure. The asymmetric unit contained two protein monomers. The space group was P41212 with cell dimensions a=b=97.38 Å, c=121.16 Å. Refmac5 was used for refinement and Coot for model building. Data in the interval 30-2.1 Å resolution were used and refined to R = 19.80% and Rfree = 24.20%. Coordinates for the crystal structure were deposited in the Protein Data Bank, with accession code 3hkv.