PARP15

PDB:3GEY

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC101701
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*sm
Host:*E.coli* BL21(DE3) R3 pRARE, where R3 denotes a derivative of BL21(DE3) resistant to a strain of T1 bacteriophage (SGC Oxford) and the pRARE plasmid originating from the Rosetta strain (Novagen) supplies tRNAs for rare codons.

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*smNLPEHWTDMNHQLFCMVQLEPGQSEYNTIKDKFTRTCSSYAIEKIERIQNAFLWQSYQVKKRQ
MDIKNDHKNNERLLFHGTDADSVPYVNQHGFNRSCAGKNAVSYGKGTYFAVDASYSAKDTYSKPDSNGRKHMYVVRVLTGVFTKGRA
GLVTPPPKNPHNPTDLFDSVTNNTRSPKLFVVFFDNQAYPEYLITFTA

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 60 ml TB supplemented with 8 g/l glycerol, 100 µg/ml kanamycin and 34 µg/ml chloramphenicol at 30 °C overnight. The overnight culture (60 ml) was used to inoculate three bottles with 1.5 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and approximately 0.2 ml PPG P2,000 81380 anti-foam solution (Fluka) per bottle. The cultures were grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 °C until OD600 reached ~2. The bottles were down-tempered to 18 °C over a period of 1 hour before target expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight and cells were harvested the following morning by centrifugation (5,500 x *g*, 10 min, 4 °C). The resulting cell pellet (65.1 g wet cell weight) was resuspended in lysis buffer (1.5 ml/g cell pellet), supplemented with 6000 U Benzonase (Merck) and 3 tablets of Complete EDTA-free protease inhibitor (Roche Applied Science). The cell suspension was stored at -80 °C.

Purification

Procedure
Columns
IMAC 1: Ni-charged 5 ml HisTrap HP (GE Healthcare)
IMAC 2: Ni-charged 1 ml HiTrap Chelating HP (GE Healthcare)
Desalting column: HiPrep 26/10 Desalting (GE Healthcare)
Gel filtration column: HiLoad 16/60 Superdex 75 Prep Grade (GE Healthcare)

Procedure
Purification of the protein was performed as a 4 step process on an ÄKTAxpress system (GE Healthcare). Prior to purification, columns were equilibrated with IMAC wash1 buffer (IMAC and desalting columns) and gel filtration buffer (GF column), respectively. The filtered lysate was loaded onto the 5 ml Ni-charged HisTrap column and washed with IMAC wash1 buffer followed by IMAC wash3 buffer. Bound protein was eluted from the IMAC column with IMAC elution buffer and automatically loaded onto the desalting column. The following day, fractions containing target protein were pooled and loaded onto the 1 ml Ni-charged HiTrap Chelating column and 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. Fractions containing the target protein were pooled and fresh TCEP was added to a final concentration of 2 mM. The protein was subsequently concentrated using a Vivaspin 20 centrifugal filter device with 10,000 MWCO (Sartorius) to 32.4 mg/ml in a volume of 1.1 ml. The identity 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 (49,100 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 using a 96-well plate. 0.1 µl of the protein solution (32.4 mg/ml) was mixed with 0.1 µl of well solution consisting of 0.1 M HEPES, pH 7.5 and 26% PEG 3350. The plate was incubated at 20 ºC and crystals appeared after 3 days. The crystal was soaked in well solution supplemented with 10 mM PJ34 for 20 hours before transferred to cryo solution consisting of 0.1 M HEPES, pH 7.5, 26% PEG3350, 0.2 M NaCl and 15% Glycerol, and flash-frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data to 2.2 Å resolution was collected at DIAMOND beamline I03.
Data Processing: The structure was solved by molecular replacement using apo PARP-15 as template (PDB: 3BLJ). The space group was P21 with cell dimensions a=45.08 Å b=137.63 Å c=68.05 Å, β=90.37°. Four monomers were located in the asymmetric unit. Pseudomerohedral twinning was observed, the twinning fraction estimated to 38%. Carefully assignment of the Rfree set of reflections so that Rwork and Rfree were not related by the twin law was performed. PHENIX was used for refinement and Coot for model building. Data in the interval 24.2-2.20 Å resolution was used and at the end of the refinement the R values were: R=21.4% and Rfree=27.0%. Coordinates for the crystal structure were deposited in the Protein Data Bank, accession code 3GEY.