PPIL2

PDB:1ZKC

Revision

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Tag:N-terminal histag with integrated thrombin cleavage site: mgsshhhhhhssglvpr*gs
Host:BL21(DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsGYVRLHTNKGDLNLELHCDLTPKTCENFIRLCKKHYYDGTIFHRSIRNFVIQGGDPTGTGTGGESYWG
KPFKDEFRPNLSHTGRGILSMANSGPNSNRSQFFITFRSCAYLDKKHTIFGRVVGGFDVLTAMENVESDPKTDRPKEEIRIDATTVF
VDPYEEADAQIAQERKTQLKIAP

Vector:p28a-LIC

Growth

Medium:TB
Antibiotics:
Procedure: Using the SGC's LEX bubbling system, PPIL2 was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 microG/mL of kanamycin at 37degC to an OD_{600} of 7.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15ºC. The culture was centrifuged and the cell pellets were collected and stored at -80degC.

Purification

Procedure
IMAC purification: 4 microL of clarified supernatant is reserved for later analysis by SDS-PAGE. The rest of the clarified supernatant is then diluted 1:2 in lysis buffer, and loaded at approximately 1mL/min by gravity onto 5 mL of Ni-NTA resin (Qiagen 30450). 5 column volumes of lysis buffer are used to wash the column at approximately 3 mL/min, followed by 5 column volumes of low imidazole buffer at approximately 3 mL/min. A 4 µL sample of the low imidazole wash is saved for later analysis by SDS-PAGE. Samples are eluted from the Ni-NTA resin by exposure to 10 mL elution buffer at 1mL/min flow rate. A 10 µL sample of the eluate is

saved for SDS-PAGE analysis. 10 microL of each eluate is saved for measurement of protein concentration using Bradford reagent (BioRad 500-0202).

Size exclusion chromatography: An XK 16x65 column (part numbers 18-1031-47 and 18-6488-01, GE Healthcare) packed with HighLoad Superdex 200 resin (10-1043-04, GE Healthcare) is pre-equilibrated with gel filtration buffer for 1.5 column volumes using an AKTAxpress (18-6645-05, GE Healthcare) at a flow rate of 3 mL/min. 5 mL of sample is loaded onto the column at 1.5 mL/min, and 2 mL fractions are collected into 96-well plates (VWR 40002-012) using peak fractionation protocols with the following parameters: (Slope; min. peak width 0.833 min; level 0.000 mAU; peak start slope 10.000 AU/min; peak end slope 20.000 AU/min). Peak fractions are analyzed for purity using SDS-PAGE or visual analysis of the chromatogram and pooled.

Concentration: Purified proteins are concentrated using either 4 mL or 15 mL concentrators with an appropriate molecular weight cut-off (Amicon Ultra-15 10,000 MWCO, UFC901024 or 5,000 MWCO, UFC900524, as appropriate, Millipore) to a final concentration of 20 mg/mL for crystallographic screening or other biophysical studies.

Extraction

Procedure
Frozen cell pellets contained in bags (Beckman 369256) obtained from 2L liters of culture are thawed by soaking in warm water for 5 minutes. Each cell pellet is resuspended in 20 mL lysis buffer and 1mL Sigma general protease inhibitor (Sigma P2714-1BTL, resuspended according to manufacturerÂ□s instructions) and then homogenized using an Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60 seconds per pellet. Cell lysis is accomplished by sonication (Virtis408912, Virsonic) on ice: the sonication protocol is 10 sec pulse at half-maximal frequency (5.0), 10 second rest, for 6 minutes total sonication time per pellet. Lysed cells are placed into centrifuge tubes (363647, Beckman Coulter) and centrifuged in a JA25.50 rotor in an Avanti J-20 XPI centrifuge (Beckman Coulter) for 20 minutes at 69,673 x g. The supernatant is decanted into a beaker, and the insoluble pellet discarded.
Concentration:
Ligand
MassSpec:
Crystallization: Purified PPIL2 was crystallized using the sitting drop vapor diffusion method. Diffracting crystals leading to the structure grew when the protein was mixed at 20 mg/mL with the reservoir solution (containing 0.8M Potassium Sodium Tartrate tetrahydrate, 0.1M Hepes pH 7.5) in a 1:1 volume ratio.
NMR Spectroscopy:
Data Collection:
Data Processing: