Entry Clone Source: TKC

Entry Clone Accession: n/a

SGC Construct ID: PIM1A-c001

GenBank GI number: gi|4505811

Entry clone accession : The expressed protein has the sequence from gi|33304198 which has the isoform change R250G compared to gi|4505811.

Expressed protein sequence:
mhhhhhhssgvdlgtenlyfqsMLLSKIN
SLAHLRAAPCNDLHATKLAPGKEKEPLES
QYQVGPLLGSGGFGSVYSGIRVSDNLPVA
IKHVEKDRISDWGELPNGTRVPMEVVLLK
KVSSGFSGVIRLLDWFERPDSFVLILERP
EPVQDLFDFITERGALQEELARSFFWQVL
EAVRHCHNCGVLHRDIKDENILIDLNRGE
LKLIDFGSGALLKDTVYTDFDGTRVYSPP
EWIRYHRYHGRSAAVWSLGILLYDMVCGD
IPFEHDEEIIGGQVFFRQRVSSECQHLIR
WCLALRPSDRPTFEEIQNHPWMQDVLLPQ
ETAEIHLHSLSPGPS

Vector: pLIC- SGC

Tags and additions: mhhhhhhssgvdlgtenlyfq*s(m). N-terminal his6 tag, TEV-protease cleavable (*)

Host : BL21(DE3)

Growth medium, induction protocol: 1 ml from a 50 ml overnight culture in LB, 100µg/ml ampicillin was used to inoculate 1 litre of LB medium containing 100µg/ml ampicillin. Cultures were grown at 37°C until they reached an OD 600 of 0.3 and then cooled to 18°C. Expression was induced for 4 hours using 1 mM IPTG at an OD 600 of 0.6. The cells were collected by centrifugation, transferred in 30 ml binding buffer to 50 ml tubes, and frozen at -20°C.

Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol.

Extraction buffer, extraction method: The frozen cells were thawed on ice. Binding buffer (plus 1 mM PMSF) was added to a final volume of 50 ml. Cells were lysed using a high pressure cell disruptor. The lysate was centrifuged at 17,000 RPM for 30 minutes and the supernatant collected for purification.

Column 1 : DEAE cellulose (DE52, Whatmann), 10 g of resin in 2.5 x 20 cm column

Buffers: Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol

Procedure: Ion exchange - Nucleic acid removal.
The resin was hydrated in 2.5M NaCl, then washed with 50 ml binding buffer prior to loading the sample. Supernatant was applied at gravity flow, followed by a wash with 50 ml binding buffer. The column flow-through was collected.

Column 2 : Ni-NTA (Qiagen), 5 ml of 50% slurry in 1.5 x 10 cm column

Buffers : Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol; **Wash buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 20 mM Imidazole, 5% glycerol; **Elution buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 50 to 250 mM Imidazole, 5% Glycerol.

Procedure: The flowthrough from column 1 was loaded by gravity flow on the Ni-NTA column. The column was then washed with 50 ml wash buffer under gravity flow. The protein was eluted by applying 5-ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150 and 250

mM); fractions were collected until essentially all protein was eluted. After elution DTT was added to a final concentration of 10 mM.

Column 3: Ion exchange Mono Q column

Buffers: A : 50 mM Hepes pH 7.5; **B :** 50 mM Hepes pH 7.5, 2 M NaCl;

Procedure: Ion exchange was performed after overnight enzymatic treatment at 4°C (see below). Partially dephosphorylated PIM1 was applied to a 1 ml MonoQ column in buffer A and eluted from the column by a linear gradient with buffer B. Non-phosphorylated PIM1 eluted at ~250 mM NaCl and was resolved from some residual singly phosphorylated PIM1 (at Ser261) which eluted at ~280 mM NaCl.

Concentration : PIM1 samples containing either unphosphorylated or singly phosphorylated protein were pooled and concentrated in Centricons (10 kDa cut off) to 10 mg/ml.

Enzymatic treatment : (Dephosphorylation and Histag cleavage) Prior to monoQ, samples containing Ni-NTA purified PIM1 were pooled and 20µg GST-lambda phosphatase and 20µg TEV protease added for overnight incubation at 4°C. Protein solution contained 10 mM DTT and 0.05 mM MnCl 2 (higher MnCl 2 concentrations caused precipitation).

Mass spec characterization : Masses of purified proteins were confirmed by LC-MS, using an Agilent LC/MSD TOF system with reversed-phase HPLC coupled to electrospray ionisation and an orthogonal time-of-flight mass analyser. Proteins were desalted prior to mass spectrometry by rapid elution off a C3 column with a gradient of 5-95% acetonitrile in water with 0.1% formic acid. The purified PIM1 protein was homogeneous and had an experimental mass of 35546 Da (unphosphorylated fraction) or 35626 (Ser261, singly phosphorylated fraction) as expected from its primary structure

Crystallisation:
***R* - 5 Complex:** Non-phosphorylated PIM1 crystals were grown at 4°C in 1.8µL sitting drops, mixing 1.2µL PIM1 (10 mg/mL in 50mM HEPES pH 7.5, 250 mM NaCl, 5 % glycerol, 10 mM DTT) with 0.6µL precipitant stock (0.12 M sodium citrate, 60 mM BisTrisPropane pH 6.5, 12% PEG 3350, 6% ethylene glycol, and 0.3% DMSO). The ruthenium half sandwich complex (*R*)- **5** was added to the protein prior to concentration from a 10 mM stock in DMSO to give a final concentration of 1 mM.

(*S*)- 6 complex: Singly phosphorylated PIM1 (ser261) crystals were grown at 4°C in 1.8µL sitting drops, mixing 1.2µL PIM1 (10 mg/mL in 50mM HEPES pH 7.5, 250 mM NaCl, 5 % glycerol, 10 mM DTT) with 0.6µL precipitant stock (0.12 M potassium thiocyanate, 60 mM BisTrisPropane pH 7.5, 12% PEG 3350, 6% ethylene glycol, and 0.3% DMSO). The ruthenium half sandwich complex (*S*)- **6** was added to the protein prior to concentration from a 10 mM stock in DMSO to give a final concentration of 1 mM.

EA72E2 Complex: Non-phosphorylated PIM1 crystals were grown at 4°C in 150 nL sitting drops, mixing 100 nL PIM1 (10 mg/mL in 50mM HEPES pH 7.5, 250 mM NaCl, 5 % glycerol, 10 mM DTT) with 50 nL precipitant stock (10% PEG 10K, 0.2M MgCl 2 , 0.1M Tris pH 7.0). The ruthenium half sandwich complex EA72E2 was added to the protein prior to concentration from a 2 mM stock in DMSO to give a final concentration of 1 mM.

Data Collection/Resolution:
***R* - 5 Complex (PDB 2 BZH):** 1.9Å **, X-ray source:** rotating anode (Rigaku FR-E SuperBright), single wavelength

(*S*)- 6 complex (PDB 2BZI): 1.9Å **, X-ray source:** Berlin synchrotron BESSY beamline BL2

EA72E2 Complex (PDB 2BZJ): 2.05Å **, X-ray source:** Swiss Light Source synchrotron beamline SLS - X10.