PTPA (PPP2R4)

PDB:2G62

Revision

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Entry Clone Accession:BC011605
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Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*sr. (note that the tag has a point mutation in the last position, M to R))
Host:

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsrNFIIPKKEIHTVPDMGKWKRSQAYADYIGFILTLNEGVKGKKLTFEYRVSEAIEKLVALLNTLD
RWIDETPPVDQPSRFGNKAYRTWYAKLDEEAENLVATVVPTHLAAAVPEVAVYLKESVGNSTRIDYGTGHEAAFAAFLCCLCKIGVL
RVDDQIAIVFKVFNRYLEVMRKLQKTYRMEPAGSQGVWGLDDFQFLPFIWGSSQLIDHPYLEPRHFVDEKAVNENHKDYMFLECILF
ITEMKTGPFAEHSNQLWNISAVPSWSKVNQGLIRMYKAECLEKFPVIQHFKFGSLLPIHPVTSG

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Residues 22-323 of hPTPA were expressed in BL21 (DE3) from the pET based vector pNIC-Bsa4 as N-terminal 6xhis fusion. A Tev protease site is present between the his tag and the protein. Colonies were grown in 20 mL of TB supplemented with 8 g/L glycerol, 50 µg/mL Kanamycin at 37°C over night. The following morning 2×10 ml of the over night cultures inoculated 2x750 mL Terrific Broth with 8 g/L glycerol in TunAir flasks. Cells were grown at 37°C until OD600 of 1.2 Â□ 2 then down-tempering to 18 °C for 1h. Expression of target protein was induced by addition of 0.5 mM IPTG at OD600 of 2- 2.5, and was allowed to continue over night.

Purification

Procedure
The protein was purified using a 1mL Hitrap chealating HP column and a Superdex 75 gelfiltration column (Amersham, UK). The 98+% pure protein (as estimated from SDS page) was

concentrated to 35.4mg/mL in 20 mM HEPES, 300 mM NaCl, 10% glycerol, 0.5 mM TCEP. Same methods were used to purify the selenomethionine labeled protein.

Extraction

Procedure
Cells were harvested by centrifugation and pellets were in 50mM Sodium-Fosfate pH 7.5, 500mM NaCl, 10% glycerol (IMAC Bind/Wash1 Buffer) supplemented with one tablet Complete EDTA-free protease inhibitor tablet per cell pellet and freezed at -80 ºC. The cells were briefly thawed in warm water and 1000 U of Benzonase was added. Cells were disrupted by High Pressure Homogenization at 10 000 PSI and samples were centrifuged for 20 minutes at 40 000×g. The soluble fraction was decanted and filtered through 0.45µm prior to loading onto the ÄKTAxpress for furher purification.
Concentration:35 mg/ml
Ligand
MassSpec: The mass spectrometry analysis showed a difference between expected and obtained mass that could be explained by a mutation of a methionine to an arginine. In addition, after selenomethionine labeling the mass increase corresponded to seven and not eight methionines being present in the protein. The construct was sequenced and the position of the methionine to arginine mutation was found at the second methionine in the N-terminal tag.
Crystallization:
NMR Spectroscopy:
Data Collection: For data collection, the crystals were flash frozen in liquid nitrogen after adding cryo solution of 20%glycerol, 2.1 M (NH4)2SO4, 0.3 M NaCl and 0.1 M sodium cacodylate (pH of drop) directly to the drop. The native x-ray data was collected at station PXI at SLS. The selenomethionine data was collected at Bessy-14.1 on a marccd 225 detector.
Data Processing: All data was processed using the programs xds and xscale. The structure of hPTPA was solved by MAD phasing using Solve that was able to identify 5 of the 7 selenium sites present in the asymmetric unit. ARP/wARP was used to build the initial model and to place solvent molecules. Refinement using refmac and manual model building using coot gave the final model consiting of residues 22-323 of hPTPA, 7 residues of the Tev-fusion, 4 sulfates and one glycerol molecules. The N-terminus was built in two conformations. The structure was refined to an R and Rfree of 15.3 % and 18 %, respectively. All residues of the model are within the most favoured or additionally allowed regions of the ramachandran plot.