PHPT1

PDB:2HW4

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC024648
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host:BL21(DE3)

Construct

Prelude:
Sequence:

MHHHHHHSSGVDLGTENLYFQSMDLALIPDVDIDSDGVFKYVLIRVHSAPRSGAPAAESKEIVRGYKWAEYHADIYDKVSGDMQKQG
CDCECLGGGRISHQSQDKKIHVYGYSMAYGPAQHAISTEKIKAKYPDYEVTWANDGY

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from glycerol stocks were grown in 20 mL of TB supplemented with 8 g/L glycerol, 50µg/mL Kanamycin at 30°C over night. The following morning 20 mL of the over night cultures were used to inoculate 1500 mL Terrific Broth with 8 g/L 87 % glycerol, 50 µg/mL Kanamycin, 100 µL BREOX (anti-foaming agent) in glass flasks in the Large Scale Expression System (LEX). Cells were grown at 37°C until OD600 of 2.5. The cultivations were down-tempered to 18 °C for 1h in water bath. Expression of target protein was induced by addition of 0.5 mM IPTG and was allowed to continue over night at 18 °C.

Purification

Procedure
Columns: HiTrap Chelating 1 mL (IMAC); HiLoadÂ□ 16/60 Superdex 75 Prep Grade (Gel filtration), all from GE Healthcare.

Purification was conducted automatically on an ÄKTA-Xpress system (GE Healthcare) operated by UNICORN software at a flow of 0.8 mL/min. Prior to purification columns were equilibrated

with IMAC Bind/Wash1 Buffer (HisTrap HP) and Gel filtration buffer (Superdex 75). The protein sample was loaded on the HisTrap HP column that was washed with IMAC Bind/Wash1 Buffer followed by IMAC Wash2 Buffer. Bound protein was eluted from the IMAC columns with 7.5 mL of IMAC Elution Buffer and loaded onto the Gel filtration column. The chromatogram from gel filtration showed one major protein peak that consisted of highly pure PHPT1A as shown by SDS-PAGE analysis. TCEP was added to the pooled protein peak to a final concentration of 2 mM. The protein was concentrated to 23 mg/mL and flash frozen in small aliquots for storage at -80ºC. Yield of pure protein per litre of culture was 6.1 mg.

Extraction

Procedure
Cells were harvested by centrifugation (WCW 15 g) and pellets were resuspended in 70 mL of lysis buffer (50 mM Sodium-Phosphate, pH 7.5, 500mM NaCl, 10% glycerol, 10 mM Imidazole, 0.5 mm TCEP and 1 tablet Complete EDTA-free protease inhibitor (Roche Biosciences)). After adding 8 µL of a 250 U/µl benzonase (Novagen) stock solution cells were disrupted by high pressure homogenization with a high-pressure homogenizer (Stansted) (2 passes) prior to centrifugation for 20 min at 49000 g in a Sorvall SS-34 rotor. The soluble fraction was decanted and filtered through a 0.45 µm filter.
Concentration:
Ligand
MassSpec:
Crystallization:PHPT1A crystallized in 1.9 M Sodium Formate and 0.1 M bis-Tris phosphate (pH 6.5). Crystals grew within two days and diffracted to 1.9 Å at the ESRF beam line ID29.
NMR Spectroscopy:
Data Collection:
Data Processing: