PTPN5A

PDB:2CJZ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_116170
Entry Clone Source: Origine
SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s(m); TEV-cleavable (*) N-terminal his6 tag.
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmSRVLQA EELHEKALDPFLLQAEFFEIPMNFVDPKE YDIPGLVRKNRYKTILPNPHSRVCLTS
PD PDDPLSSYINANYIRGYGGEEKVYIATQG PIVSTVADFWRMVWQEHTPIIVMITNIEE MNEKCTEYWPEEQVAYDGVEITVQ
KVIHT EDYRLRLISLKSGTEERGLKHYWFTSWPD QKTPDRAPPLLHLVREVEEAAQQEGPHCA PIIVHCSAGIGRTGCFIATSI
CCQQLRQE GVVDILKTTCQLRQDRGGMIQTCEQYQFV HHVMSLYEKQLSHQS

Vector:pLIC- SGC1

Growth

Medium:
Antibiotics:
Procedure: Grow starter cultures from freshly transformed colonies in 10 ml LB , 0.1 mg/ml amp. This started culture was diluted 1:1000 in fresh media and was grown at 37°C to a density of (OD600) 0.3 and than transferred to 18°C. Expression was induced at an OD 600 of 0.8 using 1 mM IPTG. Cells were harvested after 3h by centrifugation, transferred to 50-ml tubes, and frozen in liquid nitrogen.

Purification

Procedure
Column 1 : Ni affinity, HisTrap (1 ml), in AKTA-express.
The cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of Loading buffer, 10 volumes of wash buffer and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280 was automatically collected.

Column 2: SEC using AKTA-express

Column 3: HiTrap Q
Protein fraction from desalting column loaded at 1 ml/min, the column was then washed with 10 ml of buffer A and eluted with a 20-minute gradient to 50% buffer B, followed by a step to 100% buffer B.

Protein concentration: Centricons 10 kDa cut off

Extraction

Procedure
The cell pellets (20 gr wet wt) were re-suspended in 50 ml extraction buffer, and lysed by a high pressure cell disrupter. Supernatant was centrifuged for 30 minutes at 20 rpm in a JA 20 rotor
Concentration:
Ligand
MassSpec:LC- ESI -MStof confirmed the correct mass expected for this construct.
Crystallization: Crystals were grown at 4°C in 200nl sitting drops mixing 150 nl of PTPN5A (10 mg/ml in 50mM Hepes pH 7.5, 200mM NaCl ,10mM DTT) with 50 nl of a solution containing 25% PEG 3350, 0.2M LiSO4, 100 mM Bis Tris Propane pH 5.5.
Crystals were also grown in the presence of 1 mM substrate peptide (DHTGFLpTEpYVATR) using similar conditions.
NMR Spectroscopy:
Data Collection: Diffraction data were collected using a Rigaku FRE rotating anode equipped with Varimax multilayer mirrors and a Rigaku HTC detector to 2.05 Å.
Data collection was performed on flash frozen crystals at 100K, 15% glycerol was used as cryoprotectant.
Data Processing: