ITPA

PDB:2CAR

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC010138
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated thrombin protease cleavage site: mgsshhhhhhssglvprg*s(m).
Host:E. coli BL21(DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsMAASLVGKKIVFVTGNAKKLEEVVQILGDKFPCTLVAQKIDLPEYQGEPDEISIQKCQEAVRQVQGPV
LVEDTCLCFNALGGLPGPYIKWFLEKLKPEGLHQLLAGFEDKSAYALCTFALSTGDPSQPVRLFRGRTSGRIVAPRGCQDFGWDPCF
QPDGYEQTYAEMPKAEKNAVSHRFRALLELQEYFGSLAA

Vector:p28A-LIC

Growth

Medium:
Antibiotics:
Procedure:20 microL competent BL-21 cells were transformed with 1 microL plasmid. 10 min on ice, 45 sec at 42 degC. Added 100 microL SOC then 30min at 37 degC. Cells were plated on Kanamycin. Colonies were grown in 50mL of TB + 4% glycerol at 37degC, until OD600 of 0.5 then diluted into 400 mL TB + 4% glycerol. Growth at 37degC until OD600 of 1.2 - 1.5. Then at 18 degC for 1h. Induction with 0.5 mM IPTG at OD600 of 1.4 - 1.8. Left culture at 18degC over night.

Purification

Procedure
Columns: HisTrap HP 1 mL (IMAC); MonoS 5/50 GL (Cation Exchange)

Procedure: All chromatography equipment was obtained from GE Healthcare. Purification was conducted on an ÄKTA purifier 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 IEX

buffer A (MonoS). The protein sample was loaded on the HisTrap HP column, and the column was washed with IMAC Bind/Wash Buffer 1 followed by IMAC Wash Buffer 2. Bound protein was eluted from the IMAC columns with 7.5 mL of IMAC Elution Buffer. Total protein (ca. 70 mg) was concentrated to a volume of 1 mL in a Vivaspin cartridge and cleaved with 30 units of thrombin over night at room temperature. The sample was diluted 1:20 with IEX buffer A and applied to a MonoS column. The protein passing the MonoS column was essentially pure ITPA liberated from the His6-tag as judged by SDS-PAGE and TOF-MS analysis. The material eluting with IEX buffer B contained traces of uncleaved fusion protein, thrombin, as well as other contaminants. Pure ITPA protein was dialyzed into storage buffer, concentrated to 50 mg/mL and stored as aliquots at -80 ºC. Analytical gel filtration on a Superdex 75 column in storage buffer showed that the protein migrated as a dimer.

Extraction

Procedure

Concentration:
Ligand
MassSpec:
Crystallization: Crystals of the apo protein were obtained using haning drop method at 4degC. Drops were prepared using 1 µL of protein (concentration 25 mg/mL) and 1 microL of the well solution (0.2 M potassium chloride, 27 % (w/v) PEG 3350, pH 6.9). The crystals diffracted to 1.1 Å.

Crystals of the complex were obtained using sitting drops at 4degC with 900 nanoL of protein and 900 nl of well solution. The well solution contained 0.2 M potassium chloride, 31% (w/v) polyethylene glycol 3350, and the protein was added from a 50 mg/ml stock solution containing 10 mM ITP and 20 mM HEPES, pH 7.5.
NMR Spectroscopy:
Data Collection:
Data Processing: The crystal was pseudo merohedrally twinned and the structure was refined using ShelX.