PTGDS

PDB:2WWP

Revision

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Entry Clone Accession:gi|730305, BC041463, 14559
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:PTGDSA-k019
Tag:C-terminal hexahistidine tag
Host:*E.coli* BL21(DE3) R3 pRARE

Construct

Prelude:
Sequence:

MAPEAQVSVQPNFQQDKFLGRWFSAGLASNSSWLREKKAALSMCKSVVAPATDGGLNLTSTFLRKNQCETRTMLLQPAGSLGSYSYR
SPHWGSTYSVSVVETDYDQYALLYSQGSKGPGEDFRMATLYSRTQTPRAELKEKFTAFCKAQGFTEDTIVFLPQTDKCMTEQAHHHH
HH

Vector:pNIC-CH2

Growth

Medium: Cells from a glycerol stock were used to inoculate 40 mL LB supplemented with 100 µg/mL kanamycin and 34 µg/mL chloramphenicol, and grown at 30 °C overnight. 11 mL of the overnight culture was used to inoculate 0.75 L TB supplemented with 8 g/L glycerol, 50 µg/mL kanamycin and approximately 5-10 drops of Dow Corning Antifoam. The culture was grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 °C until OD600 reached ~2. The culture was down-tempered to 18 °C over a period of 1 1/2 hour before target expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight and cells were harvested the following day at noon by centrifugation (4,400 x *g*, 10 min, 4 °C). The resulting cell pellet (20 g wet cell weight) was resuspended in lysis buffer (1.5 mL/g cell pellet) supplemented with one tablets of Complete EDTA-free protease inhibitor (Roche Applied Science) and 2000 U Benzonase (Merck) per 100 mL lysis buffer, and stored at -80 °C.
Antibiotics:
Procedure:

Purification

Procedure
Columns

IMAC: Ni-charged 2 x 1 ml HiTrap Chelating HP (GE Healthcare)
Gel filtration column: HiLoad 16/60 Superdex 75 Prep Grade (GE Healthcare)

Procedure
Purification of the native protein was performed as a two step process on an ÄKTAxpress system (GE Healthcare). Prior to purification, columns were equilibrated with IMAC wash1 buffer and gel filtration buffer, respectively. The filtered lysate was loaded onto the Ni-charged HiTrap Chelating column and washed with IMAC wash1 buffer followed by IMAC wash2 buffer. Bound protein was eluted from the IMAC column with IMAC elution buffer and automatically loaded onto the gel filtration column. The target protein eluted from the size exclusion column in two peaks, one large one corresponding to a monomer and one small peak with a suggested low molecular weight. Samples of both peaks were run on an SDS PAGE gel and showed bands of equal size. However the two peaks were pooled seperately and then concentrated using an Amicon Ultra-15 centrifugal filter device, 5 000 NMWL (Millipore). The first peak was concentrated to 12 mg/mL in 0.9 mL whereas the second peak only gave 0.1 mL of 5 mg/mL. Both batches were stored at -80 ºC. Mass spectrometry was used to confirm the size of the purified protein, which was correct for both batches although they were both found to be non homogenous with other peaks present.

Extraction

Procedure
The cell suspension was thawed in a water bath. Cells were disrupted by sonication (VibraCell, Sonics) at 80% amplitude for 3 min effective time (puls 4s on, 4s off) and cell debris was removed by centrifugation (49,000 x *g*, 20 min, 4 ºC). The supernatant was decanted and stored at - 80 ºC. Before purification the cleared lysate was again thawed in a water bath and then filtered through a 0.45 µm flask filter.
Concentration:
Ligand
MassSpec:
Crystallization: Native crystals were obtained by the sitting drop vapour diffusion method in a 96-well plate. 0.1 µl of the protein solution (5 mg/mL) was mixed with 0.2 µl of well solution consisting of 0.2 M Sodium thiocyanate and 20% PEG3000. The plate was incubated at 4 ºC. Crystals of dimensions 20x70 µm had appeared after 5 days. Crystals were transferred to cryo solution containing well solution supplemented with 25% ethylene glycol and flash cooled in liquid nitrogen prior to data collection.

Cocrystals were set up with the substrate analog 9,11-Dideoxy-9α,11α-epoxymethanoprostaglandin F2α dissolved in DMSO. Crystals were obtained by the sitting drop vapour diffusion method in a 96-well plate. 0.1 µl of the protein solution (concentrated to 29 mg/mL) was mixed with 0.2 µl of well solution consisting of 0.2 M Sodium thiocyanate and 20% PEG3350. The plate was incubated at 4 ºC. Crystals of dimensions 30x80 µm had appeared after 2 days. Crystals were transferred to cryo solution containing well solution supplemented with 25% ethylene glycol and 5 mM substrate analog and flash cooled in liquid nitrogen prior to data collection.
NMR Spectroscopy:
Data Collection: Data were collected to 2.0 Å at DIAMOND (I03). The crystal belonged to space group P21 with cell parameters of a= 36.4 Å, b=56.59 Å and c=73.46 Å with a β=90.15.
Data Processing: Data were processed with iMosflm and Scala. The structure was solved by molecular replacement in Phaser using the mouse PTGDS coordinates (2CZT) as search model. Two molecules were present in the asymmetric unit. Manual building was performed in Coot and cycled with restrained refinement in REFMAC5, which resulted in the final model where R=

20.8% and Rfree=24.6%. The coordinates and structure factors were deposited in the PDB with accession code 2WWP.