GMPS

PDB:2VXO

Revision

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Entry Clone Accession:BC012178
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:GMPSA-k020
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*sm
Host:*E.coli* BL21(DE3) R3 pRARE, where R3 denotes a derivative of BL21(DE3) resistant to a strain of T1 bacteriophage (SGC Oxford) and the pRARE plasmid originating from the Rosetta strain (Novagen) supplies tRNAs for rare codons.

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*smGHHHYEGAVVILDAGAQYGKVIDRRVRELFVQSEIFPLETPAFAIKEQGFRAIIISGGPNSVY
AEDAPWFDPAIFTIGKPVLGICYGMQMMNKVFGGTVHKKSVREDGVFNISVDNTCSLFRGLQKEEVVLLTHGDSVDKVADGFKVVAR
SGNIVAGIANESKKLYGAQFHPEVGLTENGKVILKNFLYDIAGCSGTFTVQNRELECIREIKERVGTSKVLVLLSGGVDSTVCTALL
NRALNQEQVIAVHIDNGFMRKRESQSVEEALKKLGIQVKVINAAHSFYNGTTTLPISDEDRTPRKRISKTLNMTTSPEEKRKIIGDT
FVKIANEVIGEMNLKPEEVFLAQGTLRPDLIESASLVASGKAELIKTHHNDTELIRKLREEGKVIEPLKDFHKDEVRILGRELGLPE
ELVSRHPFPGPGLAIRVICAEEPYICKDFPETNNILKIVADFSASVKKPHTLLQRVKACTTEEDQEKLMQITSLHSLNAFLLPIKTV
GVQGDCRSYSYVCGISSKDEPDWESLIFLARLIPRMCHNVNRVVYIFGPPVKEPPTDVTPTFLTTGVLSTLRQADFEAHNILRESGY
AGKISQMPVILTPLHFDRDPLQKQPSCQRSVVIRTFITSDFMTGIPATPGNEIPVEVVLKMVTEIKKIPGISRIMYDLTSKPPGTTE
WE

Vector:pNIC-BSA4

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 60 ml TB supplemented with 8 g/l glycerol, 100 µg/ml kanamycin and 34 µg/ml chloramphenicol at 37 °C overnight. The overnight culture (60 ml) was used to inoculate three bottles with 1.5 l TB each, supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and approximately 0.5 ml Dow Corning anti-foam RD emulsion 63213 4D (BDH Silicone Products). The cultures were grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 °C until OD600 reached ~2. The bottles were down-tempered to 18 °C over a period of 30 minutes before target expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight and cells were harvested the following morning by centrifugation (4,400 x *g*, 20 min, 4 °C) and the resulting cell pellet (63.6 g wet cell weight) was stored at -80 °C.

Purification

Procedure
Columns
IMAC: Ni-charged 1 ml HiTrap Chelating HP (GE Healthcare)
Gel filtration column: HiLoad 16/60 Superdex 200 Prep Grade (GE Healthcare)

Procedure
Purification of the 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 divided into three samples and each was loaded onto a HiTrap Chelating column and washed with IMAC wash1 buffer followed by IMAC wash2 buffer. Bound protein was eluted from the IMAC columns with IMAC elution buffer and automatically loaded onto a gel filtration column. Fractions containing the target protein were pooled. The protein was subsequently concentrated using a Vivaspin 20 centrifugal filter device with 30,000 NMWL (Sartorius) to 13.0 mg/ml in a volume of 0.04 ml and stored at -80 ºC. The identity of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The frozen cell pellet was thawed and resuspended in lysis buffer (1.5 ml/g cell pellet), supplemented with one knife edge lysozyme, 3000 U Benzonase (Merck) and 3 tablets of Complete EDTA-free protease inhibitor (Roche Applied Science). Cells were disrupted by sonication (Vibra-Cell, Sonics) at 80% amplitude for 3 min effective time (pulsed 4s on, 4s off) repeated two times. Cell debris was removed by centrifugation (49,100 x *g*, 20 min, 4 ºC) and the supernatant was decanted and then filtered through a 0.45 µm flask filter.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained by the sitting drop vapour diffusion method in a 96-well plate. 5mM XMP, 5mM AMPPNP and 10 mM MgCl2 were added to the protein solution (13 mg/ml). 0.2 µl of the protein sample was then mixed with 0.1 µl of well solution consisting of 0.1 M sodium acetate trihydrate, pH 5.2, 1.6 M ammonium sulfate and 0.2 M sodium chloride. The plate was incubated at 20 ºC. The crystal was quickly transferred to cryo solution containing 0.1 M sodium acetate trihydrate pH 5.2, 1.6 M ammonium sulfate, 0.3 M sodium chloride and 25% glycerol, and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data to 2.5 Å resolution was collected at BESSY BL14-2.
Data Processing: The crystal belonged to space group P212121 with cell parameters 98.52, 123.42, 127.96, 90, 90, 90. Data was processed and scaled using XDS and XSCALE. The structure was solved by molecular replacement using a domain search in PHASER. As search models the glutaminase domain of human GMPS (pdb-code:2VPI) and the synthetase domain of GMPS from *T. thermophilus* (pdb-code:2YWC) were used. The initial model was then automatically built using PHENIX autobuild. Only the XMP used in crystallization was visible in the structure. Final cycles of model building and refinement were performed in Coot and REFMAC5. Data in the interval 20 - 2.5 Å resolution was used and at the end of the refinement the R values were: R= 20.6% and Rfree= 25.6%. The coordinates for the crystal structure were deposited in the Protein Data Bank, accession code 2VXO.