CTPS2

PDB:2V4U

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC034986
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:
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*smKICSIALVGKYTKLRDCYASVFKALEHSALAINHKLNLMYIDSIDLEKITETEDPVKFHEAWQ
KLCKADGILVPGGFGIRGTLGKLQAISWARTKKIPFLGVCLGMQLAVIEFARNCLNLKDADSTEFRPNAPVPLVIDMPEHNPGNLGG
TMRLGIRRTVFKTENSILRKLYGDVPFIEERHRHRFEVNPNLIKQFEQNDLSFVGQDVDGDRMEIIELANHPYFVGVQFHPEFSSRP
MKPSPPYLGLLLAATGNLNAYLQQGCKLS

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 20 ml TB supplemented with 8 g/l glycerol, 100 µg/ml kanamycin and 34 µg/ml chloramphenicol at 30 °C overnight. The overnight culture (20 ml) was used to inoculate 1.5 l TB supplemented with 50 µg/ml kanamycin and approximately 200 µl PPG P2,000 81380 anti-foam solution (Fluka). The culture was grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 °C until OD600 reached ~2.4. The bottle was down-tempered to 18 °C over a period of 1 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 morning by centrifugation (5000 x *g*, 10 min, 4 °C). The resulting cell pellet (32.4 g wet cell weight) was resuspended in lysis buffer (1.5 ml/g cell pellet), supplemented with 1500 U Benzonase (Merck) and one tablet of Complete EDTA-free protease inhibitor (Roche Applied Science). The cell suspension was stored at -80 °C.

Purification

Procedure

Columns
IMAC: Ni-charged 5 ml HiTrap Chelating HP (GE Healthcare)
Gel filtration column: HiLoad 26/60 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 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. Fractions containing the target protein were pooled and fresh TCEP was added to a final concentration of 2 mM. The protein was subsequently concentrated using an Amicon Ultra-15 centrifugal filter device with 10,000 NMWL (Millipore) to 17.9 mg/ml in a volume of 1 ml. The identity of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The cell suspension was quickly thawed in water and diluted with lysis buffer to a final volume of 2 x 68 ml. Cells were disrupted by sonication (Vibra-Cell, Sonics) at 80% amplitude for 3 min effective time (pulsed 4s on, 4s off) and cell debris was removed by centrifugation (49,000 x *g*, 20 min, 4 ºC). The supernatant was decanted and filtered through a 0.45 µm flask filter.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained by the hanging drop vapour diffusion method in a 24-well plate with 500 µl well volume. 1 µl of the protein sample (17.9 mg/ml) was mixed with 1 µl of well solution consisting of 0.1 M HEPES pH 7, 0.02 M MgCl2 and 30% (w/v) polyacrylic acid 5100. The plate was incubated at 4 ºC. To obtain a complex with the glutamine inhibitor DON, crystals were soaked for 90 min in a solution containing 0.1 M HEPES pH 7.0, 0.02 M MgCl2, 25% polyacrylic acid 5100, 0.3 M NaCl, 10% glycerol and 20.6 mM 6-Diazo-5-oxo-L-norleucine (DON) before flash frozen into liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data was collected at ESRF ID23-1.
Data Processing: The crystal belonged to space group C2 with the cell parameters 118.88, 72.33, 41.47, 90, 95.6, 90. Data was processed and scaled using XDS and XSCALE. The structure was solved by molecular replacement using MOLREP with the structure of the human glutaminase domain from CTP synthetase 2 (pdb-code:2VKT) as search model. Simulated annealing was run using PHENIX. Final cycles of model building and refinement were performed in COOT and REFMAC5. Data in the interval 20 - 2.3 Å resolution was used and at the end of the refinement the R values were R= 21.1% and Rfree= 25.8%. The coordinates for the crystal structure were deposited in the Protein Data Bank, accession code 2V4U.