AHCYL1

PDB:3MTG

Revision

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Entry Clone Accession:gi|21361647, BC016942
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:AHCYL1A-k019
Tag:N-terminal hexahistidine tag and StrepII tag with integrated TEV protease cleavage site: mhhhhhhssgwshpqfekgtrnlyfq*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:

mhhhhhhssgwshpqfekgtrnlyfq*smVSPREKQQTNSKGSSNFCVKNIKQAEFGRREIEIAEQDMSALISLRKRAQGEKPLAGA
KIVGCTHITAQTAVLIETLCALGAQCRWSACNIYSTQNEVAAALAEAGVAVFAWKGESEDDFWWCIDRCVNMDGWQANMILDDGGDL
THWVYKKYPNVFKKIRGIVEESVTGVHRLYQLSKAGKLCVPAMNVNDSVTKQKFDNLYCCRESILDGLKRTTDVMFGGKQVVVCGYG
EVGKGCCAALKALGAIVYITEIDPICALQACMDGFRVVKLNEVIRQVDVVITCTGNKNVVTREHLDRMKNSCIVCNMGHSNTEIDVT
SLRTPELTWERVRSQVDHVIWPDGKRVVLLAEGRLLNLSCSTVPTFVLSITATTQALALIELYNAPEGRYKQDVYLLPKKMDEYVAS
LHLPSFDAHLTELTDDQAKYLGLNKNGPFKPNYYRY

Vector:pNIC-NHS

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 ºC overnight. The overnight culture (20 ml) was used to inoculate 3 l TB supplemented with 8 g/l glycerol and 50 µg/ml kanamycin. The culture was grown at 37 ºC until OD_{600} reached ~1. The culture was down-tempered to 18 ºC 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*, 10 min, 4 ºC). The resulting cell pellet (46 g wet cell weight) was resuspended in lysis buffer (2 ml/g cell pellet), supplemented with 2000 U Benzonase (Merck) and one tablet of Complete EDTA-free protease inhibitor (Roche Applied Science). The cell suspension was stored at -80 ºC.

Purification

Procedure
Columns

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

Procedure

IMAC columns were equilibrated with IMAC wash1 buffer, and gel filtration columns were equilibrated with GF buffer. Purification of the protein was performed on an ÄKTA prime system (GE Healthcare). 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 loaded onto the gel filtration column. Fractions containing the target protein were identified by SDS-PAGE and pooled. The protein concentration was 3.9 mg/ml in a volume of 22.5 ml.

Tag removal

The N-terminal histidine tag and StrepII tag were proteolytically removed by incubating the target protein with His-tagged TEV protease in a molar ratio of 50:1 at 4 ºC overnight. The proteolytic reaction went to completion, as judged by SDS-PAGE. Target protein was purified from tag and protease by passing the reaction mixture over a Ni-charged 1 ml HiTrap Chelating HP column (GE Healthcare) pre-equilibrated with IMAC wash1 buffer. The protein was retrieved from flow through.The protein was concentrated and the buffer was changed to GF buffer with 2 mM TCEP using a Vivaspin 20 centrifugal filter device with 30,000 MWCO (Sartorius). The final protein concentration was determined to 33.5 mg/ml in a volume of 1.3 ml The identity of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The cell suspension was quickly thawed in water. 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 sitting drop vapour diffusion method in a 24-well plate. 0.8 µl of the protein solution (diluted to 23 mg/ml with GF buffer) was mixed with 0.8 µl of well solution consisting of 0.2 M tri-sodium citrate dehydrate, 18% PEG 3350, and 0.1 M CHES, pH 8.5. The plate was incubated at 4 ºC and crystals appeared within 5 days. The crystals were quickly transferred to a cryo solution consisting of well solution complemented with 20% (v/v) glycerol, and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data to 2.64 Å resolution was collected at BESSY (BL14-2).
Data Processing: The structure was solved by automated program BALBES. The space group was C121 with cell dimensions a=186.24 Å, b=68.6 Å, c=90.37 Å, α=90.00, β=115.27, γ=90.00. Two monomers were located in the asymmetric unit. Refmac and autoBUSTER were used for refinement and Coot for model building. Data in the interval 33.59-2.64 Å resolution was used and at the end of the refinement the R values were: R=20.42% and Rfree=24.9%. Coordinates for the crystal structure were deposited in the Protein Data Bank, accession code 3MTG.