ADSS2

PDB:2V40

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|15214463
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host:*E.coli* BL21-Gold(DE3)pRARE2, where BL21-Gold(DE3) cells (Stratagene) have been transformed with pRARE2 originating from the Rosetta2 strain (Novagen). The pRARE2 plasmid supplies tRNAs for rare codons.

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*smPRARPGGNRVTVVLGAQWGDEGKGKVVDLLAQDADIVCRCQGGNNAGHTVVVDSVEYDFHLLP
SGIINPNVTAFIGNGVVIHLPGLFEEAEKNVQKGKGLEGWEKRLIISDRAHIVFDFHQAADGIQEQQRQEQAGKNLGTTKKGIGPVY
SSKAARSGLRMCDLVSDFDGFSERFKVLANQYKSIYPTLEIDIEGELQKLKGYMEKIKPMVRDGVYFLYEALHGPPKKILVEGANAA
LLDIDFGTYPFVTSSNCTVGGVCTGLGMPPQNVGEVYGVVKAYTTRVGIGAFPTEQDNEIGELLQTRGREFGVTTGRKRRCGWLDLV
LLKYAHMINGFTALALTKLDILDMFTEIKVGVAYKLDGEIIPHIPANQEVLNKVEVQYKTLPGWNTDISNARAFKELPVNAQNYVRF
IEDELQIPVKWIGVGKSRESMIQLF

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 ºC overnight. The overnight culture (20 ml) was used to inoculate 1.5 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and approximately 200 µl BREOX FMT 30 anti-foam solution (Cognis Performance Chemicals UK Ltd). The culture was grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 ºC until OD600 reached ~2.3. The culture was down-tempered to 18 ºC 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 (4,400 x *g*, 10 min, 4 ºC). The resulting cell pellet (40.3 g wet cell weight) was resuspended in lysis buffer (1 ml/g cell pellet), supplemented with 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 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, 10,000 NMWL (Millipore) to 16.1 mg/ml in a volume of 0.35 ml. The identity of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The cell suspension was quickly thawed in water and 2000 U Benzonase (Merck) was added. 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 (49,100 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 containing 500 µl well solution. The protein was co-crystallized with 2 mM GDP. 1 µl of the protein solution (16.1 mg/ml) was mixed with 1 µl of well solution consisting of 0.1 M Tris pH 8.2, 24% PEG 2000 MME and 0.2 M trimethylamine N-oxide. The plate was incubated at 4 ºC. The crystals were quickly transferred to a cryo solution consisting of 0.1 M Tris pH 8.2, 23% PEG 2000 MME, 0.2 M trimethylamine N-oxide, 0.2 M NaCl and 20% glycerol, and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data was collected at the ESRF beamline ID14-2.
Data Processing: Data was processed with XDS in space group P6322 (a=155.4, b=155.4, c=82.1). The structure was solved with molecular replacement using PHASER. As a search model the mouse muscle adenylosuccinate synthetase (PDB-code: 1LOO) was used. Model building and refinement were performed in COOT and REFMAC5. Data in the interval 29.4-1.9 Å resolution was used and at the end of the refinement the R values were: R= 16.5% and Rfree= 19.8%. Coordinates for the crystal structure were deposited in the Protein Data Bank, accession code 2V40.