GART

PDB:2V9Y

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|4503915
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*smAARVLIIGSGGREHTLAWKLAQSHHVKQVLVAPGNAGTACSEKISNTAISISDHTALAQFCKE
KKIEFVVVGPEAPLAAGIVGNLRSAGVQCFGPTAEAAQLESSKRFAKEFMDRHGIPTAQWKAFTKPEEACSFILSADFPALVVKASG
LAAGKGVIVAKSKEEACKAVQEIMQEKAFGAAGETIVIEELLDGEEVSCLCFTDGKTVAPMPPAQDHKRLLEGDGGPNTGGMGAYCP
APQVSNDLLLKIKDTVLQRTVDGMQQEGTPYTGILYAGIMLTKNGPKVLEFNCRFGDPECQVILPLLKSDLYEVIQSTLDGLLCTSL
PVWLENHTALTVVMASKGYPGDYTKGVEITGFPEAQALGLEVFHAGTALKNGKVVTHGGRVLAVTAIRENLISALEEAKKGLAAIKF
EGAIYRKDIGFRAIAFLQQPRSLTYKESGVDIAAGNMLVKKIQPLAKATSRSGCKVDLGGFAGLFDLKAAGFKDPLLASGTDGVGTK
LKIAQLCNKHDTIGQDLVAMCVNDILAQGAEPLFFLDYFSCGKLDLSVTEAVVAGIAKACGKAGCALLGGETAEMPDMYPPGEYDLA
GFAVGAMERDQKLPHLERITEGDVVVGIASSGLHSNGFSLVRKIVAKSSLQYSSPAPDGCGDQTLGDLLLTPTRIYSHSLLPVLRSG
HVKAFAHITGGGLLENIPRVLPEKLGVDLDAQTWRIPRVFSWLQQEGHLSEEEMARTFNCGVGAVLVVSKEQTEQILRGIQQHKEEA
WVIGSVVARAEGSPRVKVKNLIESMQINGSVLKNGSLTNHFSFEKKKARVAVLISGTGSNLQALIDSTREPNSSAQIDIVISNKAAV
AGLDKAERAGIPTRVINHKLYKNRVEFDSAIDLVLEEFSIDIVCLAGFMRILSGPFVQKWNGKMLNIHPSLLPSFKGSNAHEQALET
GVTVTGCTVHFVAEDVDAGQIILQEAVPVKRGDTVATLSERVKLAEHKIFPAALQLVASGTVQLGENGK

Vector:PNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 150 ml LB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and 34 µg/ml chloramphenicol at 37 ºC overnight. The overnight culture (80 ml) was used to inoculate 4 x 750 ml TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin, 34 µg/ml chloramphenicol and approximately 200 µl BREOX FMT 30 anti-foam solution (Cognis Performance Chemicals UK Ltd). The culture was grown inTunAir flasks at 37 ºC until OD600 reached ~2. The cultures were 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 (106 g wet cell weight) 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 ÄKTAprime 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 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 a Vivaspin 20 centrifugal filter device with 10,000 MWCO (Sartorius) to 12.4 mg/ml in a volume of 0.5 ml. The identity of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The cell pellet was thawed and resuspended in lysis buffer (1 ml/g cell pellet) supplemented with a knife edge of lysozyme (Sigma), Benzonase (Merck) and two 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) and cell debris was removed by centrifugation (49,100 x *g*, 30 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. 0.2 mg/ml of chymotrypsin was added to the protein solution prior to crystallization. 1 µl of protein solution (12.4 mg/ml) was mixed with 1 µl of well solution containing, 0.1 M bis-Tris pH 5.2, 0.2 M ammonium sulfate and 27% PEG 3350. The plate was incubated at 20 ºC and crystals appeared in a couple of days. The crystal was quickly transferred to a cryo solution consisting of 0.1 M bis-Tris pH 5.2, 0.2 M ammonium sulfate and 25% PEG 3350, 0.2 M NaCl and 20% glycerol, and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data collection was carried out at BESSY BL14-1.
Data Processing:\"Data was processed with XDS in space group P21212 (a=80.67 b=80.99 c=98.33). The structure was solved with molecular replacement using PHASER. As a search model the the *E.coli* aminoimidazole ribonucleotide synthetase (PDB-code: 1CLI) was used. Model building and refinement were performed in COOT and REFMAC5. Data in the interval 15-2.1 Å resolution was used and at the end of the refinement the R values were: R= 19.4% and R free= 24% using one TLS group per molecule. The coordinates for the crystal structure were deposited in the Protein Data Bank, accession code 2V9Y. The N-teminal and C-terminal domain of GART was removed by chymotrypsin leaving only the AIRS domain. According to mass

spectrometry the size of this domain was 34968 Da, and the most probable sequence for the segment was: KVDLGGFAGL FDLKAAGFKD PLLASGTDGV GTKLKIAQLC NKHDTIGQDL VAMCVNDILA QGAEPLFFLD YFSCGKLDLS VTEAVVAGIA KACGKAGCAL LGGETAEMPD MYPPGEYDLA GFAVGAMERD QKLPHLERIT EGDVVVGIAS SGLHSNGFSL VRKIVAKSSL QYSSPAPDGC GDQTLGDLLL TPTRIYSHSL LPVLRSGHVK AFAHITGGGL LENIPRVLPE KLGVDLDAQT WRIPRVFSWL QQEGHLSEEE MARTFNCGVG AVLVVSKEQT EQILRGIQQH KEEAWVIGSV VARAEGSPRV KVKNLIESMQ INGSVLKN