GUAD

PDB:2UZ9

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession:BC053584
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(DE3) (Novagen)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*smCAAQMPPLAHIFRGTFVHSTWTCPMEVLRDHLLGVSDSGKIVFLEEASQQEKLAKEWCFKPCE
IRELSHHEFFMPGLVDTHIHASQYSFAGSSIDLPLLEWLTKYTFPAEHRFQNIDFAEEVYTRVVRRTLKNGTTTACYFATIHTDSSL
LLADITDKFGQRAFVGKVCMDLNDTFPEYKETTEESIKETERFVSEMLQKNYSRVKPIVTPRFSLSCSETLMGELGNIAKTRDLHIQ
SHISENRDEVEAVKNLYPSYKNYTSVYDKNNLLTNKTVMAHGCYLSAEELNVFHERGASIAHCPNSNLSLSSGFLNVLEVLKHEVKI
GLGTDVAGGYSYSMLDAIRRAVMVSNILLINKVNEKSLTLKEVFRLATLGGSQALGLDGEIGNFEVGKEFDAILINPKASDSPIDLF
YGDFFGDISEAVIQKFLYLGDDRNIEEVYVGGKQVVPFSSSV

Vector:PNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 20 ml TB supplemented with 8 g/l glycerol and 50 µg/ml kanamycin at 30 ºC overnight. The overnight culture (10 ml) was used to inoculate 750 ml TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin. The culture was grown in a TunAir flask at 37 ºC until OD600 reached ~1. 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 (5,500 x *g*, 10 min, 4 ºC). The resulting cell pellet (17.9 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 HisTrap 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 buffer was exchanged into 20 mM HEPES, 5 mM glycine pH 7.5 and the protein was concentrated using an Amicon Ultra-15 centrifugal filter device, 5,000 NMWL (Millipore) to 16.8 mg/ml in a volume of 1.5 ml. The identity of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The cell suspension was quickly thawed in water and 1000 U Benzonase (Merck) was added. Cells were disrupted by high-pressure homogenization (TC5-0612W-332 from Stansted fluid power LTD) 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 containing 500 µl well solution. 1 µl of the protein solution (16.8 mg/ml) was mixed with 1 µl of well solution consisting of 0.3 M Mg-formate and 11% (w/v) PEG 3350. The plate was incubated at 20 ºC and crystals appeared within 7 days. The crystals were quickly transferred to cryo solution containing 0.3 M Mg-formate, 11% (w/v) PEG 3350, 0.2 M NaCl and 25.5% glycerol and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data was collected at BESSY (BL14.2)
Data Processing:X-ray data in space group C2 (91.73, 85.71, 78.96, $\alpha = \gamma = 90$, $\beta = 116.49$) was processed using MOSFLM and SCALA. The structure was solved by molecular replacement using MOLREP with the guanine deaminase structure from *Clostridium acetobutylicum* (2I9U) as our search model. Final cycles of model building and refinement were performed in COOT and REFMAC5.