

GMDS

PDB:1T2A

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:gi:9087147

Entry Clone Source:synthetic, codon-optimized gene

SGC Clone Accession:

Tag:N-terminal: His-tag with integrated TEV protease site: mgsshhhhhssgrenlyfqghm. C-terminal: gs

Host:E.coli BL21 (Gold Magic)

Construct

Prelude:

Sequence:

```
mgsshhhhhssgrenlyfqghmRIVALITGITGDGSYLAEFLLKGYEVHGIVRRSSSFNTGRIEHLKPNQAHIEGNMKLHYGD  
LTDSTCLVKIINEVKPTEIYNLGAQSHVKISFDLAEYTADVGVGTLRLLDVAVKTCGLINSVKFYQASTSELYGKVVQEIIPQKETTPF  
YPRSPYGAALKLYAYWIVVNFREAYNLFVAVNGILFNHESPRRGANFVTRKISRVAKIYLGQLECFSLGNLDAKRDWGHAKDYVEAMW  
LMLQNDPEPDFVIATGEVHSVREFVEKSF LHIGKTIVWEGKNENEVGRCKETGKVHVTVDLKYRPTVEVDFLQGDCTKAKQKLNWKP  
RVAFDELVREMVHADVELMRTNPNAGs
```

Vector:p11

Growth

Medium:TB

Antibiotics:

Procedure:GMDSA was expressed in E. coli (BL21 Gold Magic) in Terrific Broth (TB) in the presence of 50 µg/mL of carbenicillin and kanamycin at 37 °C to an OD600 of 0.8. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.5 mM, and incubated overnight at 15 °C.

Purification

Buffers

Wash buffer: 10mM HEPES pH7.5, 0.5 M NaCl, 5% glycerol, 30 mM imidazole

Elution buffer: 10mM HEPES pH7.5, 0.5 M NaCl, 5% glycerol, 250 mM imidazole

Crystallization buffer: 10 mM HEPES, pH 7.5, 0.5 M NaCl

Procedure

The cleared lysate was loaded onto a Ni-NTA (nickel-nitrilotriacetic acid) column from Qiagen at 4degC. The column was washed with wash buffer, and the protein was eluted with elution buffer.

The purified protein was dialyzed overnight into crystallization buffer at 4degC and concentrated using Amicon Ultra centrifugal filter devices (Millipore). The protein was further purified to homogeneity by gel filtration (HighLoad 16/60 Superdex 75, Amersham Biosciences) equilibrated with crystallization buffer.

Extraction

Buffers

Binding buffer: 10 mM HEPES pH 7.5, 0.5 M NaCl, 5% glycerol, 5 mM imidazole

Procedure

Cultures were centrifuged and the cell pellets were resuspended in binding buffer with protease inhibitor (0.1 μ M benzamidine-HCl, 0.1 μ M phenylmethyl sulfonyl fluoride, PMSF) and flash frozen. The thawed cell pellet was lysed by a combination of 0.5% CHAPS (Sigma) and sonication. Lysate was cleared by centrifugation and passed through DE52 from Whatman in 0.5 M NaCl.

Concentration: 15 mg/mL

Ligand

GDP/NADPH/MassSpec:

Crystallization: Purified GMD R23-A372 was crystallized using the hanging drop vapor diffusion method. In the presence of 10mM GDP and 2mM NADPH, crystals grew when the protein (15mg/mL) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop equilibrated against a reservoir solution containing 21% PEG 4000, 0.1 M Tris pH 8.5, 0.2 M sodium acetate, and 3% dioxane or 75mM n-octyl-B-D-glucoside.

NMR Spectroscopy:

Data Collection:

Data Processing: