MGC2408

PDB:2PXX

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:14150112
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated thrombin protease site: mgsshhhhhhssglvprgs
Host:*E.coli* BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:
Sequence:

gsGYREVEYWDQRYQGAADSAPYDWFGDFSSFRALLEPELRPEDRILVLGCGNSALSYELFLGGFPNVTSVDYSSVVVAAMQAcYAH
VPQLRWETMDVRKLDFPSASFDVVLEKGTLDALLAGERDPWTVSSEGVHTVDQVLSEVSRVLVPGGRFISMTSAAPHFRTRHYAQAY
YGWSLRHATYGSGFHFHLYLMHKGGKLSVAQLALGAQILSP

Vector:pET28a-LIC

Growth

Medium:M9
Antibiotics:
Procedure:MGC2408 was expressed in E.coli BL21 (DE3) codon plus RIL in M9 minimal medium in the presence of 50 µg/ml of kanamycin at 37 °C to an OD600 of 1.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, in the presence of 50 mg/L of SeMet and incubated overnight at 15 °C.

Purification

Procedure
Column 1: 5 ml HiTrap Chelating column (Amersham Biosciences).
Column 2: Superdex200 column (26x60) (Amersham Biosciences).
Column 3: Source 30Q column (10x10) (Amersham Biosciences).
The crude extract was cleared by centrifugation. The clarified lysate was loaded onto 5 ml HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of wash buffer, and the protein was eluted with elution buffer. The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM Tris-HCl buffer, pH 8.0, and 150 mM NaCl, at flow rate 4 ml/min. Thrombin (Sigma) was added to

combined fractions containing MGC2408 and incubated overnight at 4 degC. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30Q column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM Tris-HCl, pH 8.0, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 4.5 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 12, 227 Xg. The cell pellets were frozen in liquid nitrogen and stored at -80 °C. For the purification, 11 g of the cell paste was thawed and resuspended in 110 ml lysis buffer with protease inhibitor (1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:11 mg/ml.
Ligand
MassSpec:expected MW =24079.83 Da, measured MW= 24080 Da.
Crystallization:: Purified MGC2408 was complexed with S-adenosyl-L-homocysteine (SAH) (Sigma) at 1:5 molar ratio of protein:SAH and crystallized using the hanging drop vapor diffusion method at 20 degC by mixing 1.5 µl of the protein solution with 1.5 µl of the reservoir solution containing 28% PEG3350, 0.1 M $(NH_4)_2SO_4$,0.1 M BisTris, pH 5.5.

NMR Spectroscopy:
Data Collection:
Data Processing: