MEPCE

PDB:3G07

Revision

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Revised by:created
Revision Date:created
Entry Clone Accession:NP_062552
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:

gsPLPAAGFKKQQRKFQYGNYCKYYGYRNPSCEDGRLRVLKPEWFRGRDVLDLGCNVGHLTLSIACKWGPSRMVGLDIDSRLIHSAR
QNIRHYLSEELRLPPQTLEGDPGAEGEEGTTTVRKRSCFPASLTASRGPIAAPQVPLDGADTSVFPNNVVFVTGNYVLDRDDLVEAQ
TPEYDVVLCLSLTKWVHLNWGDEGLKRMFRRIYRHLRPGGILVLEPQPWSSYGKRKTLTETIYKNYYRIQLKPEQFSSYLTSPDVGF
SSYELVATPHNTSKGFQRPVYLFHKARSPSH

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure:FLJ20257 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 degC to an OD600 of 0.8. 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 degC.

Purification

Procedure
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 onto a Superdex 200 column (26x60) (Amersham Biosciences), equilibrated in 20 mM HEPES, pH 7.4, 500 mM NaCl. Thrombin (Sigma) was added to combined fractions containing FLJ20257 and incubated overnight at 4 degC. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (Amersham Biosciences), equilibrated with 20 mM HEPES pH 7.4

buffer, and eluted with linear gradient of NaCl up to 0.5 M concentration (20CV). Purification yield was 6 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80˚C. For the purification the cell paste was thawed and resuspended in lysis buffer with protease inhibitor (1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:11.6 mg/ml.
Ligand
MassSpec: The expected mass for FLJ20257 (SeMet) is 33280.71 Da, measured mass is 33279.1053 Da.
Crystallization: Purified FLJ20257 (10 mg/mL) was complexed with S-adenosyl-L-methionine (SAM, Sigma) at 1:10 molar ratio of protein: SAM and crystallized using the hanging drop vapor diffusion method by mixing 2 microL of protein solution with 2 microL of the reservoir solution containing 22% PEG 3350, 0.1 M Ammonium Sulfate, 0.1 M Tris-HCl, pH 8.0.
NMR Spectroscopy:
Data Collection:
Data Processing: