METT10D

PDB:2H00

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:21362066
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:

gsGRVSLNFKDPEAVRALTCTLLREDFGLSIDIPLERLIPTVPLRLNYIHWVEDLIGHQDSDKSTLRRGIDIGTGASCIYPLLGATL
NGWYFLATEVDDMCFNYAKKNVEQNNLSDLIKVVKVPQKTLLMDALKEESEIIYDFCMCNPPFFANQLEAKGVNSRNPRRPPPSSVN
TGGITEIMAEGGELEFVKRIIHDSLQLKKRLRWYSCMLGKKCSLAPLKEELRIQGVPKVTYTEFCQGRTMRWALAWSFYD

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:MGC3329 protein was expressed in E.coli BL21 (DE3) codon plus RIL in M9 minimal medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37 degC to an OD600 of 0.8 and 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
Column 1: DE52 column
Column 2: 5 ml HiTrap column (Amersham Biosciences)
Column 3: Superdex200 column

The crude extract was cleared by centrifugation and passing through 20-ml DE52 column equilibrated in 20 mM Hepes, pH 7.5, containing 500 mM NaCl and 5% glycerol. The lysate was loaded onto 5 ml HiTrap column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM Hepes pH 7.5, containing 500 mM NaCl and 50 mM imidazole,

5% glycerol, and the protein was eluted with elution buffer. The protein was dialyzed against 20 mM Hepes, pH 7.5, 500 mM Ammonium Acetate, 5% glycerol, 10mM ß-mercaptoethanol. Thrombin was used for His tag cleavage during the dialysis with the present of 1mM CaCl2. The dialyzed protein was loaded on to a Superdex200 column for further purification with 20mM PIPES pH6.5, 500mM ammonium acetate. Purification yield was 11mg per liter 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 (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:16 mg/ml
Ligand
MassSpec: The expected mass for MGC3329 (SeMet) is 29121.3 Da, measured mass is 29121.8 Da.
Crystallization: Purified MGC3329 protein was complexed with S-adenosyl-L-homocysteine (SAH) (Sigma) at 1:5 molar ratio of protein:SAH and crystallized using hanging drop vapor diffusion method at 20 °C by mixing the protein solution with the reservoir solution containing 1.7M Ammonium Sulfate, 0.2M K/Na Tartrate, 0.1M Na Citrate pH 5.6.
NMR Spectroscopy:
Data Collection:
Data Processing: