FBL

PDB:2IPX

Revision

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Entry Clone Accession:GI:12056465
Entry Clone Source:MGC
SGC Clone Accession:FBL_06-A6-APC041
Tag: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQ*G
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene).

Construct

Prelude:
Sequence:

gGKNVMVEPHRHEGVFICRGKEDALVTKNLVPGESVYGEKRVSISEGDDKIEYRAWNPFRSKLAAAILGGVDQIHIKPGAKVLYLGA
ASGTTVSHVSDIVGPDGLVYAVEFSHRSGRDLINLAKKRTNIIPVIEDARHPHKYRMLIAMVDVIFADVAQPDQTRIVALNAHTFLR
NGGHFVISIKANCIDSTASAEAVFASEVKKMQQENMKPQEQLTLEPYERDHAVVVGVYRP

Vector:pET28a-MHL

Growth

Medium:
Antibiotics:
Procedure:FBL protein was expressed in E.coli BL21 (DE3) codon plus RIL in 12L M9 minimal medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37oC 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 15oC.

Purification

Procedure
The crude extract was cleared by centrifugation. 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 7.4, containing 500 mM NaCl 5% glycerol and 50 mM imidazole, and the protein was eluted with elution buffer (20 mM HEPES, pH 7.4, containing 500 mM NaCl, 5% glycerol, and 250 mM imidazole). The eluted protein was pooled and dialyzed overnight against 20 mM HEPES pH7.4, 500 NaCl, 10% Glycerol, 10mM ß-mercaptoethanol, in the presence of TEV protease to remove His-tag. The cleaved protein was separated from uncleaved protein on a 5-ml Ni HiTrap column. Purification yield was 2 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at ~75000 x g for 60 minutes. The cell pellets were frozen in liquid nitrogen and stored at -80°C. For purification, the cell paste was thawed and resuspended in lysis buffer (50 mM HEPES, pH 7.4, 0.5 M NaCl, 5 mM imidazol, 2 mM ß-mercaptoethanol, 5% glycerol) with protease inhibitor (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through a Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:26.8 mg/ml
Ligand
MassSpec: The expected mass for FBL(SeMet) is 25996.56Da, measured mass is 25996.9874 Da.
Crystallization: Purified FBL protein (10.7 mg/ml) was complexed with 5Â□-methylthioadenosine (MTA) (Sigma) at 1:5 molar ratio of protein:MTA and crystallized using sitting drop vapor diffusion method at 20 °C by mixing the protein solution with the reservoir solution containing 20% PEG3350, 0.2 M CaCl2.
NMR Spectroscopy:
Data Collection:
Data Processing: