MYST1

PDB:2PQ8

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:14149875
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 (Stratagene).

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsTKVKYVDKIHIGNYEIDAWYFSPFPEDYGKQPKLWLCEYCLKYMKYEKSYRFHLGQCQWRQPPGKEIY
RKSNISVHEVDGKDHKIYCQNLCLLAKLFLDHrTLYFDVEPFVFYILTEVDRQGAHIVGYFSKEKESPDGNNVACILTLPPYQRRGY
GKFLIAFSYELSKLESTVGSPEKPLSDLGKLSYRSYWSWVLLENLRDFRGTLSIKDLSQMTSITQNDIISTLQSLNMVKYWKGQHVI
CVTPKLVEEHLKSAQYKKPPITVDSVCLKWAPPK

Vector:pET28a-LIC

Growth

Medium:TB
Antibiotics:
Procedure:MYST1 was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin. Cell were grown at 37oC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, incubated overnight at 15oC.

Purification

Procedure
The crude extract was cleared by centrifugation and passing through 20-ml DE52 column equilibrated in 20 mM HEPES, pH 7.4, containing 1 M NaCl and 5% glycerol. The lysate was loaded onto 10 ml Chelating Sepharose column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM HEPES buffer, pH 7.4, containing 0.5 M NaCl , 50 mM imidazole, 5% glycerol and 0.1% CHAPS, and the protein was eluted with elution buffer (20 mM HEPES, pH 7.4, 0.5 M NaCl, 250 mM imidazole, 5% glycerol, 0.1 % CHAPS). The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with

20 mM HEPES buffer, pH 7.4, and 0.5 M NaCl, at flow rate 4 ml/min. Purification yield was 8.2 mg of the protein per 1L of culture.
Enzymatic treatment: Thrombin

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 (50 mM HEPES, pH 7.4, 0.5 M NaCl, 5 mM imidazol, 2 mM β-mercaptoethanol, 5% glycerol, 0.1% CHAPS) 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:6.8 mg/ml
Ligand
MassSpec: The expected mass for MYST1 is 34364.5 Da, measured mass is 34275.5 Da.
Crystallization: Purified MYST1 was complexed with acetylcoenzyme A (AcCoA, Sigma) at 1:10 molar ratio of protein:AcCoA and crystallized using the sitting drop vapor diffusion method at 20 °C by mixing 1µl of the protein solution with 1 µl of the reservoir solution containing 20% PEG3350, 0.2 M CaCl2.
NMR Spectroscopy:
Data Collection:
Data Processing: