SMYD3

PDB:3QWP

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:19570495
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQG
Host:*E.coli* BL21 (DE3) codon plus RIL (Stratagen)

Construct

Prelude:
Sequence:

gMEPLKVEKFATANRGNGLRAVTPLRPGELLFRSDPLAYTVCKGSRGVVCDRCLLGKEKLMRCSQCRVAKYCSAKCQKKAWPDHKRE
CKCLKSCKPRYPPDSVRLLGRVVFKLMDGAPSESEKLYSFYDLESNINKLTEDrKEGLRQLVMTFQHFMREEIQDASQLPPAFDLFE
AFAKVICNSFTICNAEMQEVGVGLYPSISLLNHSCDPNCSIVFNGPHLLLRAVRDIEVGEELTICYLDMLMTSEERRKQLRDQYCFE
CDCFRCQTQDKDADMLTGDEQVWKEVQESLKKIEELKAHWKWEQVLAMCQAIISSNSERLPDINIYQLKVLDCAMDACINLGLLEEA
LFYGTRTMEPYRIFFPGSHPVRGVQVMKVGKLQLHQGMFPQAMKNLRLAFDIMRVTHGREHSLIEDLILLLEECDANIRAS

Vector:pET28-MHL

Growth

Medium:M9 minimal medium
Antibiotics:50 μg/ml of kanamycin
Procedure:SMYD3 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 °C to an OD_{600} 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 °C.

Purification

Procedure
The crude extract was cleared by centrifugation. The clarified lysate was loaded onto 5 ml HiTrap Chelating column (Amersham Biosciences), charged with Ni^{2+}. 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. 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 6 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 degrees Celsius. 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:15 mg/ml
Ligand
MassSpec: Expected MW = 49920.25 Da
Measured MW = 49920.7725 Da
Crystallization: Purified SMYD3 (10 mg/mL) was complexed with S-adenosyl-L-methionine (SAM) (Sigma) at 1:10 molar ratio of protein:SAM and crystallized using the sitting drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution with 1 µl of the reservoir solution containing 20% PEG 3,350, 0.2 M calcium chloride.
NMR Spectroscopy:
Data Collection:
Data Processing: