Human SRM in complex with MTA

PDB:2O05

Revision

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Revised by:created
Revision Date:created
Entry Clone Accession:gi4507208
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:

gsMEPGPDGPAASGPAAIREGWFRETCSLWPGQALSLQVEQLLHHRRSRYQDILVFRSKTYGNVLVLDGVIQCTERDEFSYQEMIAN
LPLCSHPNPRKVLIIGGGDGGVLREVVKHPSVESVVQCEIDEDVIQVSKKFLPGMAIGYSSSKLTLHVGDGFEFMKQNQDAFDVIIT
DSSDPMGPAESLFKESYYQLMKTALKEDGVLCCQGECQWLHLDLIKEMRQFCQSLFPVVAYAYCTIPTYPSGQIGFMLCSKNPSTNF
QEPVQPLTQQQVAQMQLKYYNSDVHRAAFVLPEFARKALNDVS

Vector:p28a-LIC

Growth

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

Purification

Procedure
The clarified lysate was loaded onto a 5-ml HiTrap Chelating column from Amersham, charged with Ni2+, on AKTA FPLC system. The column was washed with 5 CV of wash buffer (20 mM HEPES, pH 7.4, 500 mM NaCl, 50 mM Imidazole, 5% glycerol), and the protein was eluted with 5 CV of elution buffer (20mM HEPES, pH 7.4, 500 mM NaCl, 5% glycerol, 250 mM imidazole). The protein was loaded onto a gel filtration column (26X60 Superdex200, Amersham Biosciences) equilibrated with buffer containing 20 mM TrisHCl, pH 8.0, 150 mM NaCl. The purified protein was treated with thrombin (Sigma) overnight at 4ºC. The protein was further

purified to homogeneity by ion-exchange chromatography on a 20-ml Source 30Q column, (Amersham Biosciences) equilibrated with buffer containing 20 mM TrisHCl, pH 8.0 and eluted with linear gradient of NaCl up to 500 mM concentration.

Extraction

Procedure
Cultures were centrifuged. The cell pellets were flash frozen in liquid nitrogen and stored at -80 ºC. For purification, the cell pellet was thawed and resuspended in lysis buffer (50 mM HEPES pH 7.4, 0.5 M NaCl, 5% glycerol, 5 mM imidazole, 0.1µM phenylmethyl sulfonyl fluoride (PMSF), 0.1% CHAPS). Cells were lysed by passing through Microfluidizer (Microfluidics). Lysate was clarified by centrifugation and passing through DE52 column (Whatman).
Concentration:25.2 mg/ml
Ligand
MassSpec:
Crystallization: Purified SRM protein was crystallized in the presence of dcAdoMet, MTA, MTA and putrescine, MTA and spermidine, using the hanging drop vapor diffusion method at 20°C by mixing equal volume of the protein solution with the reservoir solution. SRM-MTA complex was crystallized in 20% PEG3350, 0.2 M Mg(oAC)2; SRM-dcAdoMet complex in 25% PEG3350, 0.2 M MgCl2, 0.1 M Tris-HCl, pH 8.5. SRM-MTA-putrescine ternary complex was crystallized in 20% PEG3350, 0.2 M ammonium formate; SRM-MTA-spermidine ternary complex in 25% PEG3350, 0.1 M (NH4)2SO4, 0.1 M HEPES-NaOH pH 7.5.
NMR Spectroscopy:
Data Collection:
Data Processing: