SMS

PDB:3C6M

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:21264341
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:

gsRHSTLDFMLGAKADGETILKGLQSIFQEQGMAESVHTWQDHGYLATYTNKNGSFANLRIYPHGLVLLDLQSYDGDAQGKEEIDSI
LNKVEERMKELSQDSTGRVKRLPPIVRGGAIDRYWPTADGRLVEYDIDEVVYDEDSPYQNIKILHSKQFGNILILSGDVNLAESDLA
YTRAIMGSGKEDYTGKDVLILGGGDGGILCEIVKLKPKMVTMVEIDQMVIDGCKKYMRKTCGDVLDNLKGDCYQVLIEDCIPVLKRY
AKEGREFDYVINDLTAVPISTSPEEDSTWEFLRLILDLSMKVLKQDGKYFTQGNCVNLTEALSLYEEQLGRLYCPVEFSKEIVCVPS
YLELWVFYTVWKKAKP

Vector:pET28a-LIC

Growth

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

Purification

Procedure
The crude extract was cleared by centrifugation and loaded onto 5 ml HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM Tris-HCl buffer, pH 8.0, containing 250 mM NaCl and 50 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.0, 250 mM NaCl, 250 mM imidazole, 5% glycerol). 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. Thrombin (Sigma) was added to combined fractions containing SMS and incubated

overnight at 4oC. 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 2.3 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 purification the cell paste was thawed and resuspended in lysis buffer (phosphate-buffered saline, pH 7.4, 0.25 M NaCl, 5 mM imidazol, 2 mM β-mercaptoethanol, 5% glycerol) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:21.8 mg/ml
Ligand
MassSpec:expected MW = 41067.95 Da, measured MW = 41055.637 Da.
Crystallization: Purified was complexed with spermine and 5Â□-methylthioadenosine (protein: SPM:MTA ratio at 1:5:5) and crystallized using the hanging drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution with 1 µl of the reservoir solution containing18 % PEG 20000, 0.1 M NaCl, 0.1 M BisTris, pH 6.5.
NMR Spectroscopy:
Data Collection:
Data Processing: