SMS

PDB:3C6K

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_004586
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:
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 37 degC to OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15 degC.

Purification

Procedure
Column 1: 5 ml HiTrap Chelating column (Amersham Biosciences)
Column 2: Superdex200 column (26x60) (Amersham Biosciences)
Column 3: Source 30Q column (10x10) (Amersham Biosciences)

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 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. Thrombin (Sigma) was added to combined fractions containing SMS and incubated overnight at 4 degC. 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.

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80 degC. For purification the cell paste was thawed and resuspended in lysis buffer 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 spermidine and 5Â□-methylthioadenosine 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 containing12 % PEG 20000, 0.1 M MES, pH 6.5.
NMR Spectroscopy:
Data Collection:
Data Processing: