TSG101

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Tag:N-termlnal tag: MGSSHHHHHHSSGLVPRGS
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsSLISAVSDKLRWRMKEEMDRAQAELNALKRTEEDLKKGHQKLEEMVTRLDQEVAEVDKNIELLKKKDE
ELSSALEK

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure: Competent BL21 (DE3) cells (Invitrogen C6000-03) were transformed and grown using the LEX system (Harbinger BEC) at 37 degC in 2L bottles (VWR 89000-242). In order to obtain the selenomethionyl derivative of the TSG101 domain, the cells were grown in M9 medium supplemented with supplemented with 150 mM glycerol, 100 µg/ml Kanamycin and 600 µl antifoam 204 (Sigma A-8311) using a M9 SeMET High-Yield growth media kit package (Medicilon MD045004-50L) according to manufacturer's instruction. When OD600 0.8 - 1.2 was reached, the temperature was reduced to 20 degC, and one hour later protein expression was induced with 100 µM IPTG (BioShop IPT001) and the culture was incubated overnight (16

hours) at 20 degC. Cell pellets were collected by centrifugation (12,227 x g, 20 min), frozen and stored at -80 degC.

Purification

Procedure
The cleared lysate was loaded onto a 3 mL TALON metal-affinity resin column (Clontech Laboratories 635504) at 4ºC. The column was washed with 10 mL Wash Buffer A, 10 mL Wash Buffer B and 10 mL Wash Buffer A. The protein was eluted with 6 mL Elution Buffer. The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare 17-1069-01) using Gel Filtration Buffer. Fractions containing SeMet TSG101 were pooled and concentrated by ultrafiltration. The N-terminal His-tag was removed by overnight incubation of the protein with thrombin (1 unit/mg protein) at 4°C, and final protein purification was achieved by anion-exchange chromatography on a 5-ml HiTrapQ HP column (GE Healthcare 17-1154-01) using Ion-Exchange Buffer A and a 0 - 0.5 M NaCl gradient created by mixing Buffer A with Ion-Exchange Buffer B. The yield of the protein was 6 mg per liter of bacterial culture; its purity (SDS-PAGE) was about 98 %. Molecular weight of the product (9181.2, LC/MS) indicated that all 3 methionyls were substituted with selenomethionyls.

Extraction

Procedure
After resuspension in 30 mL per liter bacterial culture of Lysis Buffer, cells were lysed using a Microfluidics M110-EH microfluidizer at 18,000 psi.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals of the selenomethionyl derivative of the TSG101 domain were grown at 291 K using the hanging drop method by mixing equal volumes of protein solution (14 mg/ml) and Crystallization Buffer (2.4 M ammonium sulfate, 0.1 M bis-Tris, pH 5.5 and 1 mM dithiothreitol). The crystals were cryoprotected by immersion in well solution mixed in an 1 : 1 ratio with water solution of and placed in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data from a crystal of the selenomethionyl derivative of the TSG101 domain were collected at beamline APS-19ID of the GM/CA-CAT at the Advanced Photon Source (Argonne National Laboratory). The data set was integrated and scaled using the XDS program package.
Data Processing: The structure was solved using programs SHELXD and SHELXE. Iterative model building using the graphics program COOT and refinement package BUSTER led to a model with an R factor of 18.8 % (Rfree 21.8 %) for data between 50 and 2.59 Å.