MAGI1

PDB:3BPU

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:n/a
Entry Clone Source: Synthetic
SGC Clone Accession:
Tag:N-terminal TEV-cleavable (at *) his-tag with the following sequence mhhhhhhssgvdlgtenlyfq*s
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

sMELITVHIVKGPMGFGFTIADSPGGGGQRVKQIVDSPRSRGLKEGDLIVEVNKKNVQALTHNQVVDMLVESPKGSEVTLLVQRQTR
L

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: An overnight culture (10 ml) was used to innoculate 1L TB medium (supplemented with 50 µg/ml of Kanamycin). The cells were cultured at 37°C with vigorous shaking (160 rpm) until the culture reached an OD600 of 1.5. At that point temperature was reduced to 18°C, and cells were induced with IPTG at a concentration of 0.5 mM, and cultured further for 18 hours. Cells were harvested at 6000 rpm for 10 minutes and the cell pellet of 1L was resuspended in 20 ml of lysis buffer and stored at -20°C until further use. Lysis buffer : 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 5 mM imidazole, Complete TM EDTA-free protease inhibitor (Roche, 1tablet / 50ml).

Purification

Procedure

Column 1: Ni-NTA

Buffers: Binding buffer: 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5,

5 mM imidazole, 0.5mM TECP; Wash Buffer: 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 30 mM imidazole, 0.5mM TECP; Elution Buffer: 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 250 mM imidazole, 0.5mM TECP.

Procedure: A 0.5ml NiNTA column was equilibrated with 12 ml of Binding buffer. The lysed sample was applied to the column twice and washed through with 12 ml of Binding Buffer (Wash 1) and 25ml of Wash Buffer (Wash 2). The protein was eluted with 15mls of Elution buffer

Column 2: Size Exclusion Chromatography (SEC) Hiload 16/60 Superdex 200 prep grade 120 ml (GE/Amersham Biosciences)

Buffers: Gelfiltration bufffer: 10 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 0.5mM TCEP.

Procedure: AKTA Purifier Gel Filtration. The eluted fractions from the Ni-NTA column were loaded on the gel filtration column in Gelfiltration buffer at 1ml/min. The flow rate was 1ml/min and the pure protein was eluted at 65-82min. The protein was collected in a 96 well block and analyzed by SDS-PAGE. Positive fractions were pooled for TEV cleavage.

TEV cleavage: The gel filtration fractions containing MAGI1A were pooled and 150µl of TEV protease solution (9mg/ml) was added. The digestion was left overnight at 4°C and cleavage was examined by SDS page, before passing the mixture through Ni-NTA resin.

Extraction

Procedure
The resuspended pellet was thawed and lysed in a high pressure homogeniser and then centrifuged at 4°C for 45 minutes at 4.8g.
Concentration: The combined samples from the SEC column (identified by SDS PAGE) were concentrated using centricons with 5 kDa cut off (Amicon Ultra 5k, Millipore) to 2 mg/ml.
Ligand
MassSpec:LC-ESI-MS tof confirmed the correct mass of 9537 Da expected for this construct of MAGI1A
Crystallization: Crystals were grown by vapor diffusion at 20°C in 300nl sitting drops. The drops were prepared by mixing 200nl of protein solution and 100nl of precipitant consisting of 0.05M Zn acetate pH 6.2 and 30% PEG 3350. Crystals were transferred to a cryo-protectant consisting of the well solution and 25% glycerol before flash-cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Resolution: 1.6Å; X-ray source: SLS beam X10SA
Data Processing: