RG9MTD2

PDB:4FMW

Revision

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Entry Clone Accession:GI:197927261
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SGC Clone Accession:RG9MTD2:APC005_4-E05:C15877
Tag:N-terminal: His-tag with integrated thrombin protease site:MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:
Sequence:

gsPNSDGHDRKRVRRDVVHSTLRLIIDCSFDHLMVLKDIKKLHKQIQRCYAENRRALHPVQFYLTSHGGQLKKNMDENDKGWVNWKD
IHIKPEHYSELIKKEDLIYLTSDSPNILKELDESKAYVIGGLVDHNHHKGLTYKQASDYGINHAQLPLGNFVKMNSRKVLAVNHVFE
IILEYLETRDWQEAFFTILPQRKG

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure:RG9MTD2 was expressed in E.coli BL21(DE3) codon plus RIL in M9 minimal medium in the presence of 50 µg/ml ofkanamycin at 37°C to an OD600 of 0.8. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, in the presence of 50 mg/L ofSeMet and incubated overnight at 15°C .

Purification

Procedure
The crude extract was cleared by centrifugation and passingthrough a DE52 column. The clarified lysate was loaded onto 5 ml HiTrap Chelatingcolumn (GE Healthcare), charged with Ni2+. The column was washed with 20 CV of 20mM HEPES, pH 7.4, containing 500 mM NaCl and 50 mM imidazole, 5% glycerol, andthe protein was eluted with elution buffer (20 mM HEPES, pH 7.4, 500 mM NaCl, 250mM imidazole, 5% glycerol). The protein was dialyzed against 20 mM HEPES, pH 7.4,500 mM NaCl, 5% glycerol. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (Amersham Biosciences),equilibrated with buffer 20 mM HEPES pH 7.4, and eluted with linear gradient of

NaClup to 500 mM concentration (20CV). Purification yield was 4 mg of the protein per 1Lof culture.

Enzymatic treatment: thrombin

Extraction

Procedure
Cells were harvested by centrifugation at7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80°C. For thepurification the cell paste was thawed and resuspended in lysis buffer (50 mM HEPES,pH 7.4, 500 mM NaCl, 5 mM imidazol, 2 mM ß-mercaptoethanol, 5% glycerol) withprotease inhibitor (1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysedby passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:37.9 mg/ml
Ligand
MassSpec:expected MW= 23291.4 Da, measured MW= 23291.8 Da.
Crystallization: Purified RG9MTD2 (10 mg/mL) was complexed with S-adenosyl-L-homocystein (SAH, Sigma) at 1:5 molar ratio of protein: SAH and crystallized usinghanging drop vapor diffusion method by mixing 2 µl of protein solution with 2 µl of thereservoir solution containing 2.22 M NH4SO4; 0.1 M Tris-HCl pH8.5, 10% Glycerol.
NMR Spectroscopy:
Data Collection:
Data Processing: