TARBP1

PDB:2HA8

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:19743836
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 (Stratagen)

Construct

Prelude:
Sequence:

gsNSRVSDLDLELLFQDRAARLGKSISRLIVVASLIDKPTNLGGLCRTCEVFGASVLVVGSLQCISDKQFQHLSVSAEQWLPLVEVK
PPQLIDYLQQKKTEGYTIIGVEQTAKSLDLTQYCFPEKSLLLLGNEREGIPANLIQQLDVCVEIPQQGIIRSLNVHVSGALLIWEYT
RQQLLSHGDTKP

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:TARBP1 protein 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 37degC to an OD600 of 0.8 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15degC.

Purification

Procedure
The crude extract was cleared by centrifugation. The lysate was loaded onto 5 ml HiTrap column (Amersham Biosciences), charged with Ni^{2+}. The column was washed with 10 CV of 20 mM Tris HCl, pH 8.0, containing 250 mM NaCl and 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 TARBP1 and incubated overnight at 4degC. 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 34.5 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 -80degC. For purification, the cell paste was thawed and resuspended in lysis buffer (1X phosphate-buffered-saline, 0.25 M NaCl, 5 mM imidazol, 2 mM ß-mercaptoethanol, 5% glycerol) with protease inhibitor (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:17.7 mg/ml
Ligand
MassSpec:
Crystallization: Purified TARBP1 protein was complexed with S-adenosyl-L-homocysteine (SAH) (Sigma) at 1:5 molar ratio of protein:SAH and crystallized using sitting drop vapor diffusion method at 20 °C by mixing the protein solution with the reservoir solution containing 20% PEG 5KMME, 0.1 M Bis-Tris pH 6.5.
NMR Spectroscopy:
Data Collection:X-ray diffraction data were collected to 1.6 Å at the 17-ID beam at the APS.
Data Processing: