

COMTD1

PDB:2AVD

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:GI:31542747

Entry Clone Source:MGC

SGC Clone Accession:

Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHSSGLVPRGS

Host:E.coli BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:

Sequence:

gsPPWGRGRREQCLLPPPEDSRLWQYLLSRSMREHPALRSRLLTLEQPQGDSSMMTCEQAQLLANLARLIQAKKALDLGFTGYSALAL
ALALPADGRVVTCEVDAQPPELGRPLWRQAEAEHKIDLRLKPALETLDDELLAAGEAGTFDVAVVDADKENCAYYERCLQLLRPGGI
LAVLRLWRGKVLQPPKGDVAAECVRNLNERIRRDVRYISLLPLGDGLTLAFKI

Vector:p28a-LIC

Growth

Medium:

Antibiotics:

Procedure:COMTD1 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 37oC to OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15oC.

Purification

Procedure

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 20 mM Tris-HCl buffer, pH 8.0, containing 250 mM NaCl and 50 mM imidazole, and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.0, 250 mM NaCl, 250 mM imidazole). 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 COMTD1 and incubated overnight at 4oC. 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 2.5 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 -80°C. For purification the cell paste was thawed and resuspended in lysis buffer (phosphate-buffered saline, pH 7.4, 0.25 M NaCl, 5 mM imidazol, 2 mM β -mercaptoethanol, 5% glycerol) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.

Concentration:

Ligand

MassSpec:

Crystallization: Purified COMTD1 was complexed with S-adenosyl-L-methionine (SAM) (Sigma) at 1:10 molar ratio of protein:SAM and crystallized using the sitting drop vapor diffusion method at 20 °C by mixing 1 μ l of the protein solution with 1 μ l of the reservoir solution containing 20% PEG 3350, 0.2M Ammonium dihydrogen phosphate.

NMR Spectroscopy:

Data Collection:

Data Processing: