DMAP1

PDB:3HM5

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_061973
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQG
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene).

Construct

Prelude:
Sequence:

gGKDYPFARFNKTVQVPVYSEQEYQLYLHDDAWTKAETDHLFDLSRRFDLRFVVIHDRYDHQQFKKRSVEDLKERYYHICAKLANVR
AVPGTD

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure: The SANT domain of DMAP1 was expressed in *E.coli* BL21 (DE3) codon plus in Terrific Broth medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37 degC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM and incubated overnight at 15 degC.

Purification

Procedure
The lysate was loaded onto 5 ml HiTrap column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of wash buffer, and the protein was eluted with elution buffer. The protein was then loaded on to a Superdex200 (60X60, Amersham Biosciences) column equilibrated in 20 mM PIPES, pH 6.5 buffer containing 250 mM NaCl. TEV protease was added to combined fractions containing DMAP1. The cut and uncut protein of DMAP1 were separated on a Ni column. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM PIPES, pH 6.5, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 4.5 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 the purification the cell paste was thawed and resuspended in lysis buffer with protease inhibitor (1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:11.5 mg/ml
Ligand
MassSpec: Expected MW is 11196.5 Da, measured mass is 11196.0 Da.
Crystallization: Purified SANT domain of DMAP1 was crystallized using hanging drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution (10 mg/ml) with 1 µl of the reservoir solution containing 20% PEG 3350, 0.2 M CaCl2, 0.1 M HEPES, pH 7.0. Crystals were frozen in liquid nitrogen using glycerol as cryoprotectant.
NMR Spectroscopy:
Data Collection:
Data Processing: