SULT1C3

PDB:2REO

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_001008743
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal His-tag with TEV cleavage site:
MHHHHHHSSGRENLYFQ*G
Host:*E. coli* BL21(DE3) codon plus RIL (Stragagen)

Construct

Prelude:
Sequence:

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MAKIEKNAPTMEKKPELFNIMEVDGVPTLILSKEWWEKVANFQAKPDDLILATYPKSGTTWMHEILDMILNDGDVEKCKRAQTLDRH
AFLELKFPHKEKPDLEFVLEMSSPQLIKTHLPSHLIPPSIWKENCKIVYVARNPKDCLVSYYHFHRMASFMPDPQNLEEFYEKFMSG
KVVGGSWFDHVKGWWAAKDMHRILYLFYEDIKKDPKREIEKILKFLEKDISEEILNKIIYHTSFDVMKQNPMTNYTTLPTSIMDHSI
SPFMRKGMPGDWKNYFTVAQNEEFDKDYQKKMAGSTLTFRTEI
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Vector:pET28a-MHL

Growth

Medium:TB
Antibiotics:
Procedure:SULT1C3 was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/mL of kanamycin at 37 degC to an OD600 of 0.8. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.5 mM, and incubated overnight at 15 degC.

Purification

Procedure
Column 1: HiTrap Chelating column (Amersham Biosciences)
Column 2: Superdex200 column (26x60) (Amersham Biosciences).
Column 3: Source 30Q column (10x10) (Amersham Biosciences)
The clarified lysate was loaded onto 5 mL HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM Tris, pH 8.0, containing 250 mM NaCl and 5% glycerol, and the protein was eluted with elution buffer. The protein was

loaded onto Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM Tris buffer, pH 8.0, and 150 mM NaCl, at flow rate 4 mL/min. TEV was added to combined fractions containing SULT1C3, then dialyzed in 20 mM Tris pH 8.0, 250 NaCl, 10% glycerol, and 10 mM β-mecaptoethanol at 4 degC overnight. The protein was passed through the 5mL Ni column again with 20 mM Tris, pH 8.0, containing 250 mM NaCl and 5% glycerol, and eluted with 20 mM Tris, pH 8.0, 250 mM NaCl, 5% glycerol and 250 mM imidazole. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30Q column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM Tris buffer, pH 8.0, and eluted with linear gradient of NaCl up to 500 mM concentration (30CV). Purification yield was 3 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 degC. For the purification the cell paste was thawed and resuspended in lysis buffer with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 18,000 psi.
Concentration:22.8 mg/mL
Ligand
MassSpec:expected MW is 35913.5,
measured MW is 35913.51
Crystallization:8 mg/mL purified SULT1C3 was mixed with 2 mM 3'-phosphoadenosine 5'-phosphate (PAP, Sigma) and 2 mM N-(2-Fluorenyl)acetamide (Sigma) in 20 mM Tris buffer, pH 8.0, incubated on ice for 30 mins. SULT1C3-PAP was crystallized using hanging drop vapor diffusion method at 20°C by mixing 2 µl of the protein mix with 2 µl of the reservoir solution containing 18% polyethylene glycol 2000, 0.2 M Li di-ammonium tartrate, 0.1M MES buffer, pH 6.5, and 5% Ethylene glycol.
NMR Spectroscopy:
Data Collection:
Data Processing: