SULT1C2

PDB:2GWH

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi: 28830308
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:

gsEDFTFDGTKRLSVNYVKGILQPTDTCDIWDKIWNFQAKPDDLLISTYPKAGTTWTQEIVELIQNEGDVEKSKRAPTHQRFPFLEM
KIPSLGSGLEQAHAMPSPRILKTHLPFHLLPPSLLEKNCKIIYVARNPKDNMVSYYHFQRMNKALPAPGTWEEYFETFLAGKVCWGS
WHEHVKGWWEAKDKHRILYLFYEDMKKNPKHEIQKLAEFIGKKLDDKVLDKIVHYTSFDVMKQNPMANYSSIPAEIMDHSISPFMRK
GAVGDWKKHFTVAQNERFDEDYKKKMTDTRLTFHFQF

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure:SULT1C2 was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin at 37oC 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 15oC.

Purification

Procedure
The clarified lysate was loaded onto 5 ml HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 50 mM HEPES-NaOH, pH 7.4,, containing 500 mM NaCl and 50 mM imidazole, and the protein was eluted with elution buffer (50 mM HEPES-NaOH, pH 7.4,, 500 mM NaCl, 250 mM imidazole). The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM MES-NaOH buffer, pH 6.5, and 250 mM NaCl, at flow rate 4 ml/min. Thrombin (Sigma) was added to combined fractions containing SULT1C2 and incubated overnight at 4oC. The protein was further

purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM MES-NaOH buffer, pH 6.5, and eluted with linear gradient of NaCl up to 500 mM concentration (30CV). Purification yield was 56 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 6,000 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 (50 mM HEPES-NaOH, pH 7.4, 0.5 M NaCl, 5 mM imidazol, 2 mM ß-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:11.7 mg/ml
Ligand
MassSpec:expected MW is 34965.1, measured MW is 34965.6
Crystallization:10 mg/mL purified SULT1C2 was mixed with 2mM 3'-phosphoadenosine 5'-phosphate (PAP, Sigma) and 2mM pentachlorophenol (PCP, Sigma) in 20 mM MES-NaOH buffer, pH 6.5, and incubated on ice for 30 mins. SULT1C2-PAP-PCP complex was crystallized using the sitting drop method at 20°C by mixing 0.8 µl of the protein-cofactor-inhibitor mix with 0.8 µl of the reservoir solution containing 25% polyethylene glycol 3350, 0.2 M Li Sulfate, 0.1M Bis-Tris pH 6.5.
NMR Spectroscopy:
Data Collection:
Data Processing: