NAT1_mutant F125S

PDB:2IJA

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:42741671
Entry Clone Source:MGC
SGC Clone Accession:NAT1_14::C233A_F125S:F10-APC046
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene).

Construct

Prelude:
Sequence:

gsgsgsDIEAYLERIGYKKSRNKLDLETLTDILQHQIRAVPFENLNIHCGDAMDLGLEAIFDQVVRRNRGGWCLQVNHLLYWALTTI
GFETTMLGGYVYSTPAKKYSTGMIHLLLQVTIDGRNYIVDAGsGRSYQMWQPLELISGKDQPQVPCVFRLTEENGFWYLDQIRREQY
IPNEEFLHSDLLEDSKYRKIYSFTLKPRTIEDFESMNTYLQTSPSSVFTSKSFCSLQTPDGVHCLVGFTLTHRRFNYKDNTDLIEFK
TLSEEEIEKVLKNIFNISLQRKLVPKHGDRFFTI

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:NAT1 was expressed in E.coli BL21 (DE3) codon plus RIL in 12L M9 minimal medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37oC to an OD600 of 0.8 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, in the presence of 50 mg/L of SeMet and incubated overnight at 15oC.

Purification

Procedure
The crude extract was cleared by centrifugation at ~75000 x g for 60 minutes. The lysate was loaded onto 10 ml Chelating Sepharose column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM Tris-HCl buffer, pH 8.0, containing 500 mM NaCl , 20 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 250 mM imidazole, 5% glycerol). Eluted protein was treated with iodoacetamide. 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. 20 units of Thrombin (Sigma) was added to combined fractions containing NAT1 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 8.5, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 1.6 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation. The cell pellets were frozen in liquid nitrogen and stored at -80°C. For the purification the cell paste from 12L of cells was thawed and resuspended in lysis buffer (20 mM Tris HCl, pH 8.0, 500 mM NaCl, 5 mM imidazol, 2 mM ß-mercaptoethanol, 5% glycerol, 0.1% CHAPS) 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:4.4 mg/ml
Ligand
MassSpec: The expected mass for NAT1 is 34431.82 Da, measured mass is 34432.1363 Da.
Crystallization: Purified NAT1_F125S was crystallized using the hanging drop vapor diffusion method at 20 °C by mixing 1µl of the protein solution with 1 µl of the reservoir solution containing 26% PEG4000, 0.2 M NaAc, 0.1 M Tris HCl, pH 7.8.
NMR Spectroscopy:
Data Collection:
Data Processing: