NNMT

PDB:2IIP

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:66933018
Entry Clone Source:MGC
SGC Clone Accession:NNMT_01:K100A:E101A:E103A:D7-GBC003
Tag: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene)

Construct

Prelude:
Sequence:

```
MESGFTSKDTYLSHFNPRDYLEKYYKFGSRHSAESQILKHLLKNLFKIFCLDGVKGDLLIDIGSGPTIYQLLSACESFKEIVVTDYS
DQNLQELEKWLKaaPaAFDWSPVVTYVCDLEGNRVKGPEKEEKLRQAVKQVLKCDVTQSQPLGAVPLPPADCVLSTLCLDAACPDLP
TYCRALRNLGSLLKPGGFLVIMDALKSSYYMIGEQKFSSLPLGREAVEAAVKEAGYTIEWFEVISQSYSSTMANNEGLFSLVARKLS
RPL
```

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:NNMT mutant K100A/E101A/E103A was expressed in E.coli BL21 (DE3) codon plus RIL in 6L Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin at 37oC to an OD600 of 1.0. Cells were then 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 at ~75000 x g for 60 minutes. The clarified lysate was loaded onto 3 ml Ni-NTA column (Qiagen). The column was washed with 5 CV of 50 mM Tris-HCl buffer, pH 8.0, containing 500 mM NaCl, 5% glycerol and 25 mM imidazole, and the protein was eluted with elution buffer (50 mM Tris-HCl, pH 8.0, 500 mM NaCl, 250 mM imidazole, 5 % glycerol). The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 50 mM Tris-HCl buffer, pH 8.0, and 500 mM NaCl, 5% glycerol, 1 mM DTT, at flow rate 3.5 ml/min. Combined fractions containing NNMT mutant

K100A/E101A/E103A were dialyzed against 4 L of 50 mM Tris, pH 8.0, 100 mM NaCl, 5% glycerol, 1 mM DTT overnight at 4oC. The protein was further purified to homogeneity by passing through HiTrap CaptoQ 5 ml column (Amersham Biosciences), equilibrated with buffer 50 mM Tris-HCl, pH 8.0, 5% glycerol, 1 mM DTT. The desired protein was in unbound protein fraction. Purification yield was 12.8 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 6L of cells was thawed and resuspended in lysis buffer (50 mM Tris, pH 8.0, 0.5 M NaCl, 5 mM imidazol, 2 mM ß-mercaptoethanol, 5% glycerol) with protease inhibitor (1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:17.2 mg/ml
Ligand
MassSpec:expected MW is 31427.0 Da, measured MW is 31372.09 Da
Crystallization: Purified NNMT was complexed with 2.5 mM S-adenosyl-L-homocysteine (SAH) (Sigma) and crystallized using the sitting drop vapor diffusion method at 18 °C by mixing 0.5 µl of the protein solution with 0.5 µl of the reservoir solution containing 2.15 M ammonium sulfate, 0.1 M HEPES/Na, pH 7.2.
NMR Spectroscopy:
Data Collection:
Data Processing: