NMT1

PDB:3IU2

Revision

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Entry Clone Accession:
Entry Clone Source:
SGC Clone Accession:NMT1:MAC03F-B03:C208079
Tag:N-terminal tag: mgsshhhhhhssgrenlyfqg
Host:BL21-(DE3)-V2R-pRARE2

Construct

Prelude:
Sequence:

TMEEASKRSYQFWDTQPVPKLGEVVNTHGPVEPDKDNIRQEPYTLPQGFTWDALDLGDRGVLKELYTLLNENYVEDDDNMFRFDYSP
EFLLWALRPPGWLPQWHCGVRVVSSRKLVGFISAIPANIHIYDTEKKMVEINFLCVHKKLRSKRVAPVLIREITRRVHLEGIFQAVY
TAGVVLPKPVGTCRYWHRSLNPRKLIEVKFSHLSRNMTMQRTMKLYRLPETPKTAGLRPMETKDIPVVHQLLTRYLKQFHLTPVMSQ
EEVEHWFYPQENIIDTFVVENANGEVTDFLSFYTLPSTIMNHPTHKSLKAAYSFYNVHTQTPLLDLMSDALVLAKMKGFDVFNALDL
MENKTFLEKLKFGIGDGNLQYYLYNWKCPSMGAEKVGLVLQ

Vector:pET15-MHL

Growth

Medium:TB
Antibiotics:
Procedure:NMT1 was expressed in *E. coli* BL21-(DE3)-V2R-pRARE2 resistant strain in Terrific Broth (TB) in the presence of ampicillin/chloramphenicol (100 µg/mL and 34 µg/mL respectively). A single colony was inoculated into 100mL of LB with of ampicillin/chloramphenicol (100 µg/mL and 34 µg/mL respectively) in a 250 mL baffled flask and incubated with shaking at 250 rpm overnight at 37 °C. The culture was transferred into 900mL of TB with Carbennicillin/chloramphenicol (100 µg/mL and 34 µg/mL respectively) and 0.5 mL of antifoam (Sigma) in a 1 L bottle and cultured using the LEX system to an OD600 of 5-6, cooled to 15 °C, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 °C.

Purification

Procedure
The cleared lysate was loaded onto a 1.0-2.5 mL Ni-NTA (Qiagen) open column (pre-equilibrated with Binding Buffer) at approximately 1.5-2.0 mL/min. The Ni-NTA column was then washed

with 150 mL of Wash Buffer at 2-2.5 mL/min. After washing, the protein was eluted with Elution Buffer. The eluted sample was applied to a Sephadex S200 26/60 gel filtration column pre-equilibrated with Gelfiltration Buffer on a AKTA explorer system. The fractions corresponding to the eluted protein peak were pooled and further treated with TEV protease overnight to cut the histag. The mixture was loaded onto another 1.0mL Ni-NTA open column and the cut protein was collected from the flow through. Its identity and purity was evalulated by mass spectroscopy and SDS-PAGE gel and then concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The final concentration of the NMT1 was 5.6 mg/ml and stored at 4 ºC. For long term storage, the protein was flash frozen and stored at -80 ºC.

Extraction

Procedure
The culture was harvested by centrifugation. Pellets from 1 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with the addition of protease inhibitors (EDTA-free, Complete, Roche). Resuspended pellets stored at -80 ºC were thawed overnight at 4 °C on the day before purification. Prior to sonication, each pellet from 1 L of culture was pretreated with 0.5 % CHAPS and 500 units of benzonase for 40 minutes at room temperature. After 6 minutes sonication, the cell lysate was centrifuged using a Beckman JLA-16.250 rotor at 16,000 rpms for 45 minutes at 4 ºC.
Concentration: The final concentration of the NMT1 was 5.6 mg/ml and stored at 4 °C.
Ligand
MassSpec:
Crystallization: Inhibitor DDD90096 was disolved in DMSO to 100 mM in stock. NMT1 was incubated with 1 mM Myristol Co-enzyme A for 30 minutes on ice before adding DDD90096 to 1mM final concentration. The mixture of NMT1-MCoA-DDD90096 was co-crystallized using hanging drop vapor diffusion. Crystallization buffer is: 19% PEG3350, 0.2M di-ammonium hydrogen citrate, pH5.7 at 20degC.
NMR Spectroscopy:
Data Collection:
Data Processing: