DNMT1

PDB:3EPZ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_001370
Entry Clone Source:dnmt1.BC126227.OBS.MHS1768-98980929.pCR-XL-TOPO
SGC Clone Accession:dnmt1.0351.0600.145C02 (SDC145C02)
Tag:N-terminal: MHHHHHHSSGRENLYFQG
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

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MHHHHHHSSGRENLYFQGPKCIQCGQYLDDPDLKYGQHPPDAVDEPQMLTNEKLSIFDANESGFESYEALPQHKLTCFSVYCKHGHL
CPIDTGLIEKNIELFFSGSAKPIYDDDPSLEGGVNGKNLGPINEWWITGFDGGEKALIGFSTSFAEYILMDPSPEYAPIFGLMQEKT
YISKIVVEFLQSNSDSTYEDLINKIETTVPPSGLNLNRFTEDSLLRHAQFVVEQVESYDEAGDSDEQPIFLTPCMRDLIKLAGVTLG
QRRAQAR
```

Vector:pET28MHL

Growth

Medium:
Antibiotics:
Procedure: The protein was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin at 37 °C to an OD600 of 7.5. Protein expression was induced 0.05 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 °C. The culture was centrifuged (12,000 x g, 15 minutes) and cell pellets collected and stored at -80 °C. In order to obtain the selenomethionyl derivative of the DNMT1 RFTS-SRA-binding domain, the cells were grown in M9 medium supplemented with glycerol using a M9 SeMET High-Yield growth media kit package (MD045004-50L, Medicilon) according to manufacturer's instructuion and the protein was purified as below.

Purification

Procedure
The cleared lysate was loaded onto a 3 mL TALON metal-affinity resin column (BD Biosciences) at 4 ºC. The column was washed with 10 mL Wash buffer A, 10 mL Wash buffer B, and 10 mL Wash buffer A. The protein was eluted with 6 mL Elution buffer.

The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with Gel Filtration buffer. Fractions containing protein corresponding to the DNMT1 peak were pooled and diluted with Ion Exchange buffer A to a final NaCl concentration of 30 mM.

Final purification was achieved by ion-exchange chromatography on a 5-ml HiTrapQ column using linear 0 - 50 % gradient of Ion-exchange buffer B in Ion-exchange buffer A. The target protein eluted around 20% buffer B. The corresponding fractions were combined and protein was concentrated by ultrafiltration using an Amicon Ultra centrifugal filter with 10 kD cutoff to a final concentration of 21 mg/ml.

The yields of the protein and its selenomethionyl derivatives were approx. 1.5 and 1 mg per liter of bacterial culture, respectively.

Extraction

Procedure
The cell pellet from a 2 L culture was resuspended in 50 ml Lysis buffer, lysed using a Microfluidizer at 18,000 PSI, and cleared by centrifugation at 40,000 x g for 30 min.
Concentration:21 mg/ml.
Ligand
MassSpec: Mass-spectroscopy by LCMS showed the product molecular weight was 12 Da less than calculated for the selenomethionyl derivative of the DNMT1 domain with five methionine residues substituted with selenomethionine, 30,385 Da.
Crystallization: The selenomethionyl derivative of the DNMT1 domain was mixed with detergent Anapoe80 solution (10%, v/v) in 5:1 ratio (final protein and detergent concentrations were 16.5 mg/ml and 1.7%, respectively) and crystals were grown at 298 K using the hanging drop method by mixing 1 volume of this solution with 1 volume of well solution consisting of 23% polyethyleneglycol 3350, 0.1 M bis-Tris, pH 6.0, 0.3M Na acetate and 5 mM TCEP. The crystals were cryoprotected by immersion in the well solution mixed in 1:1 ratio with a water solution containing 20% (w/v) sucrose, 4% (w/v) glucose, 18% (v/v) glycerol and 18% (v/v) ethylene glycol and placed in liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: