PRMT3

PDB:3SMQ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:
SGC Clone Accession:GI:44771198
Tag:N-terminal: His-tag with integrated thrombin protease site:MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:
Sequence:

DLQEDEDGVYFSSYGHYGIHEEMLKDKIRTESYRDFIYQNPHIFKDKVVLDVGCGTGILSMFAAKAGAKKVLGVDQSEILYQAMDII
RLNKLEDTITLIKGKIEEVHLPVEKVDVIISEWMGYFLLFESMLDSVLYAKNKYLAKGGSVYPDICTISLVAVSDVNKHADRIAFWD
DVYGFKMSCMKKAVIPEAVVEVLDPKTLISEPCGIKHIDCHTTSISDLEFSSDFTLKITRTSMCTAIAGYFDIYFEKNCHNRVVFST
GPQSTKTHWKQTVFLLEKPFSVKAGEALKGKVTVHKNKKDPRSLTVTLTLNNSTQTYGLQ

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure: The overexpression of the recombinant protein in E. coli BL21 (DE3)pRARE2-V2R was induced by addition of 1 mM isopropyl-1-thio-D-galactopyranoside (IPTG) and overnight incubation at 15 oC.

Purification

Procedure
After clarification of the crude extract by centrifugation (16,000 rpm for onehour using Aventi-J 20-XPI, Beckman Coulter), the lysate was loaded ontoDE52 ion-exchange resin and passed through a Ni-NTA column. The columnwas washed and protein was eluted by addition of 20 mM Tris buffer, pH7.5, 500 mM NaCl, 5% glycerol, containing 30 mM and 250 mM imidazole,respectively. Protein sample was purified further using 6mL Resource QAnion exchange column (Code No:17-1179-01 , GE Healthcare) and ÄKTAFPLC (GE Healthcare) where the column was pre-equilibrated with 10 mMTris buffer, pH 7.6, followed by 10 mM Tris buffer, pH 7.6 with 1 M NaCland then re-equilibrated with 10 mM Tris buffer, pH 7.6 . The

diluted protein sample (final NaCl concentration of 50 mM) was loaded onto a 6 mL ResourceQ column .The Contaminant proteins bind and pure PRMT3 would be in flow throw with an approximate purity of 95% purity. After adjusting the NaCl final concentration to 150 mM, the protein was concentrated, flash-frozen and storedat -80 oC.

Extraction

Procedure
Harvested cells were re-suspended in 20 mM Tris buffer, pH 7.5, with 500mM NaCl, 5 mM imidazole and 5% glycerol. The cells were lysed chemically followed by sonication at a frequency of 8.5 with ten seconds on and ten seconds off (Sonicator 3000, Misoni).
Concentration: Purified protein was concentrated using 15 mL concentrators with anappropriate molecular weight cut-off (Amicon Ultra-15 10,000 MWCO,Millipore) to a final value of 2.2 mg/mL.
Ligand
MassSpec:
Crystallization:PRMT3 was incubated at 1.1 mg/mL overnight with benzo[d][1,2,3]thiadiazol-6-yl)-3-(2-cyclohexenylethyl)urea (compound 1) at 1:30 molar ratio (PRMT3:compound 1). Following incubation, protein was concentrated to 3 mg/mL and crystallized using the sitting drop diffusion method at 20°C by mixing 1 µL of the protein solution with 1 µL of the reservoir solution containing 20% PEG 4K, 0.2 M MgOAc, 0.1 M NaCaco (pH 6.5). Prior to freezing, 0.1 µL of 100 mM compound 1 was added directly to the drop. Crystals were soaked for 30 min in the same buffer with 10% glycerol.
NMR Spectroscopy:
Data Collection:
Data Processing: