RNMT

PDB:3EPP

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:4506567
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene).

Construct

Prelude:
Sequence:

gsSQSRIFYLRNFNNWMKSVLIGEFLEKVRQKKKRDITVLDLGCGKGGDLLKWKKGRINKLVCTDIADVSVKQCQQRYEDMKNRRDS
EYIFSAEFITADSSKELLIDKFRDPQMCFDICSCQFVCHYSFESYEQADMMLRNACERLSPGGYFIGTTPNSFELIRRLEASETESF
GNEIYTVKFQKKGDYPLFGCKYDFNLEGVVDVPEFLVYFPLLNEMAKKYNMKLVYKKTFLEFYEEKIKNNENKMLLKRMQALEPYPA
NESSKLVSEKVDDYEHAAKYMKNSQVRLPLGTLSKSEWEATSIYLVFAFEKQQ

Vector:pET28a-LIC

Growth

Medium:TB
Antibiotics:
Procedure:RNMT was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 μg/ml of kanamycin. Cell were grown at 37oC to an OD600 of 1.5 and 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 and passing through 20-ml DE52 column equilibrated in 20 mM HEPES, pH 7.4, containing 500 mM NaCl and 5% glycerol. The lysate was loaded onto 5 ml HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM HEPES buffer, pH 7.4, containing 500 mM NaCl , 50 mM imidazole and 5% glycerol, and the protein was eluted with elution buffer (20 mM HEPES, pH 7.4, 500 mM NaCl, 250 mM imidazole, 5% glycerol). The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM HEPES buffer,

pH 7.4, and 250 mM NaCl, at flow rate 4 ml/min. Thrombin (Sigma) was added to combined fractions containing RNMT and incubated overnight at 4oC. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM PIPES, pH 6.5, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 8.3 mg of the protein per 1L of culture.
Enzymatic treatment: Thrombin

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80˚C. For the purification the cell paste was thawed and resuspended in lysis buffer (50 mM HEPES, pH 7.4, 500 mM NaCl, 5 mM imidazol, 2 mM β-mercaptoethanol, 5% glycerol) with protease inhibitor (1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:36 mg/ml
Ligand
MassSpec: The expected mass for RNMT is 36810.26 Da, measured mass is 36811.9162 Da.
Crystallization: Purified RNMT was complexed with sinefugin (Sigma) at 1:2 molar ratio of protein:SAH and crystallized using the sitting drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution with 1 µl of the reservoir solution containing20% PEG 3,500, 0.2 M KSCN.
NMR Spectroscopy:
Data Collection:
Data Processing: