SIRT5 + Suramin

PDB:2NYR

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI: 23408
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 (Stratagen).

Construct

Prelude:
Sequence:

gsARPSSSMADFRKFFAKAKHIVIISGAGVSAESGVPTFRGAGGYWRKWQAQDLATPLAFAHNPSRVWEFYHYRREVMGSKEPNAGH
RAIAECETRLGKQGRRVVVITQNIDELHRKAGTKNLLEIHGSLFKTRCTSCGVVAENYKSPICPALSGKGAPEPGTQDASIPVEKLP
RCEEAGCGGLLRPHVVWFGENLDPAILEEVDRELAHCDLCLVVGTSSVVYPAAMFAPQVAARGVPVAEFNTETTPATNRFRFHFQGP
CGTTLPEALA

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure:SIRT5 was expressed in E.coli BL21 (DE3) codon plus RIL in TB medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37oC to an OD600 of 0.8 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. The lysate was loaded onto 5 ml HisTrap column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 30 mM HEPES-NaOH buffer, pH 7.5, containing 500 mM NaCl, 500mM Urea, 50 mM imidazole, 5% glycerol. After that protein was eluted with elution buffer (30 mM HEPES-NaOH buffer, pH 7.5, 500mM Urea, 500mM NaCl, 250 mM imidazole and 5% glycerol). The protein was loaded onto a gel filtration column (Superdex200, 26X60, Amersham Biosciences) equilibrated with buffer 30 mM HEPES-NaOH buffer, pH 7.5, containing 500 mM NaCl at flow rate 4ml/min. The

purified protein was treated with thrombin (Sigma) overnight at 4ºC. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (Amersham Biosciences), equilibrated with 30 mM HEPES-NaOH buffer, pH 7.5 and eluted with linear gradient of NaCl up to 500 mM concentration (30CV). Purification yield was 56 mg of the protein per 1L of culture.

Stock Concentration: 28.5 mg/ml

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 (1xPBS, pH 7.4, 0.25 M NaCl, 5 mM imidazol, 5% glycerol, 0.1% IPTG) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:
Ligand
MassSpec: Expected MW is 29386.5, measured mass is 29386.
Crystallization: Purified SIRT5 was complexed with Suramin (Biomol) at 1:5 molar ratio of protein: Suramin, concentrated up to 10 mg/ml and crystallized using hanging drop vapor diffusion method drop at 20°C by mixing 1.5 µl of the protein solution with 1.5 µl of the reservoir solution containing 25% PEG 3350, 0.1M Bis-Tris pH 6.5, 0.2M Na Chloride.
NMR Spectroscopy:
Data Collection:
Data Processing: