ACAT1

PDB:2F2S

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:31563501
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:

mgsshhhhhhssglvprgsEVVIVSATRTPIGSFLGSLSLLPATKLGSIAIQGAIEKAGIPKEEVKEAYMGNVLQGGEGQAPTRQAV
LGAGLPISTPCTTINKVCASGMKAIMMASQSLMCGHQDVMVAGGMESMSNVPYVMNRGSTPYGGVKLEDLIVKDGLTDVYNKIHMGS
CAENTAKKLNIARNEQDAYAINSYTRSKAAWEAGKFGNEVIPVTVTVKGQPDVVVKEDEEYKRVDFSKVPKLKTVFQKENGTVTAAN
ASTLNDGAAALVLMTADAAKRLNVTPLARIVAFADAAVEPIDFPIAPVYAASMVLKDVGLKKEDIAMWEVNEAFSLVVLANIKMLEI
DPQKVNINGGAVSLGHPIGMSGARIVGHLTHALKQGEYGLASICNGGGGASAMLIQKL

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:ACAT1 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 dialyzed

against 20 mM HEPES buffer, pH 7.4, containing 500 mM NaCl , and 5% glycerol, overnight. Purification yield was 9.3 mg of the protein per 1L of culture.

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 ß-mercaptoethanol, 5% glycerol) with protease inhibitor (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:10.5 mg/mL
Ligand
MassSpec: The expected mass for ACAT1 is 42658.2 Da, measured mass is 42528.1 Da.
Crystallization: Purified ACAT1 was complexed with acetylcoenzyme A (AcCoA) (Sigma) at 1:10 molar ratio of protein:AcCoA 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 containing 25% PEG 3350, 0.2 M Ammonium Acetate, 0.1M Bis-Tris pH 6.5.
NMR Spectroscopy:
Data Collection:
Data Processing: