ACAA1 - Human peroxisomal acetyl-CoA acyl transferase 1

PDB:2IIK

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC000635
Entry Clone Source:MGC
SGC Clone Accession:
Tag: Tag sequence: mhhhhhhssgvdlgtenlyfq*s(m) , TEV-cleavable (*), N-terminal his6 tag
Host: Rosetta-R3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmAPQASA ADVVVVHGRRTAICRAGRGGFKDTTPDEL LSAVMTAVLKDVNLRPEQLGDICVGNV
LQ PGAGAIMARIAQFLSDIPETVPLSTVNRQ CSSGLQAVASIAGGIRNGSYDIGMACGVE SMSLADRGNPGNITSRLMEKEKAR
DCLIP MGITSENVAERFGISREKQDTFALASQQK AARAQSKGCFQAEIVPVTTTVHDDKGTKR SITVTQDEGIRPSTTMEGLAK
LKPAFKKD GSTTAGNSSQVSDGAAAILLARRSKAEEL GLPILGVLRSYAVVGVPPDIMGIGPAYAI PVALQKAGLTVSDVDIFE
INEAFASQAAY CVEKLRLPPEKVNPLGGAVALGHPLGCTG ARQVITLLNELKRRGKRAYGVVSMCIGTG MGAAAVFEYPGN

Vector:pNIC28-Bsa4

Growth

Medium:TB + 50 µg/ml Kanamycin + 34 µg/ml chloramp.
Antibiotics:
Procedure:2 x 1 liter TB in 2.5-L baffled flasks were inoculated with 10 ml overnight culture and grown at 37°C. The protein expression was induced with 0.5 mM IPTG at OD600= 3.2 at 18°C overnight. The cells were collected by centrifugation and frozen at -80°C.

Purification

Procedure
Lysis buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP.
Wash buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 50 mM imidazole, 0.5 mM TCEP.
Elution buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 350 mM imidazole, 0.5 mM TCEP.
Gel filtration buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP
Column 1: Ni-affinity, HisTrap, 1 ml (GE/Amersham Biosciences)

The cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280 was automatically collected.

Column 2 : Gel filtration, Hiload 16/60 Superdex 200 prep grade, 120 ml (GE/ Amersham Biosciences)
The eluted fractions from the Ni-affinity Histrap column were loaded on the gel filtration column at 1.0 ml/min. Eluted proteins were collected in 2 ml fractions.

Concentration : The protein was concentrated in Amicon (5 K) to 25.1 mg/ml and the protein concentration determined spectrophotometrically using the predicted molar extinction coefficient 43935(M-1cm-1).

Extraction

Procedure
Frozen cell pellets were thawed at 37°C and resuspended in a total volume of 100 ml lysis buffer. The cells were disrupted by high pressure (20 kpsi) followed by sonication. Nucleic acids and cell debris were removed by adding 0.15% PEI , followed by centrifugation for 30 minutes at 40,000xg. The supernatant was further clarified by filtration (0.45 µm).
Concentration:
Ligand
MassSpec: Two peaks of 43849.5 and 43937.2 Da were detected for ACAA1A p001, which are 68.5 and 19.2 Da higher than the expected mass of 43918 Da for the his-tagged protein.
Crystallization: Crystals were grown by vapor diffusion at 20°C from a sitting drop consisting of 150 nl protein (25.1 mg/ml), containing 2 mM CaCl2 and 10 mM acetylcoenzyme A, and 150 nl well solution. The drop was equilibrated against well solution containing 25% PEG 3350, 0.24 M ammonium sulfateand 100 mM HEPES pH 7.5. The crystal was transferred to a cryoprotectant composed of 20% ethylene glycol before flash-cooling in liquid nitrogen .
NMR Spectroscopy:
Data Collection:
Data Processing: