ACADVL

PDB:2UXW

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|4557235
Entry Clone Source: Invitrogen
SGC Clone Accession:ACADVLA-c004
Tag:N-terminal TEV-cleavable (at *) his-tag with the following sequence
mhhhhhhssgvdlgtenlyfq*s
Host:BL21(DE3)-R3 / pRARE2

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsMSFAVGMFKGQLTTDQVFPYPSVLNEEQTQFLKELVEPVSRFFEEVNDPAKNDALEMVEETTWQG
LKELGAFGLQVPSELGGVGLCNTQYARLVEIVGMHDLGVGITLGAHQSIGFKGILLFGTKAQKEKYLPKLASGETVAAFCLTEPSSG
SDAASIRTSAVPSPCGKYYTLNGSKLWISNGGLADIFTVFAKTPVTDPATGAVKEKITAFVVERGFGGITHGPPEKKMGIKASNTAE
VFFDGVRVPSENVLGEVGSGFKVAMHILNNGRFGMAAALAGTMRGIIAKAVDHATNRTQFGEKIHNFGLIQEKLARMVMLQYVTESM
AYMVSANMDQGATDFQIEAAISKIFGSEAAWKVTDECIQIMGGMGFMKEPGVERVLRDLRIFRIFEGTNDILRLFVALQGCMDKGKE
LSGLGSALKNPFGNAGLLLGEAGKQLRRRAGLGSGLSLSGLVHPELSRSGELAVRALEQFATVVEAKLIKHKKGIVNEQFLLQRLAD
GAIDLYAMVVVLSRASRSLSEGHPTAQHEKMLCDTWCIEAAARIREGMAALQSDPWQQELYRNFKSISKALVERGGVVTSNPLGF

Vector:pNIC28-Bsa4

Growth

Medium:TB
Antibiotics:
Procedure: An overnight culture (10 ml) was used to innoculate 1L TB medium (supplemented with 50 µg/ml of Kanamycin). The cells were cultured in 10 litres at 37°C with vigorous shaking (160 rpm) until the culture reached an OD600 of 1.5. At that point temperature was reduced to 18°C, and cells were induced with IPTG at a concentration of 0.5 mM, and cultured further for 18 hours. Cells were harvested at 6000 rpm for 10 minutes and the cell pellet of 1L was resuspended in 20 ml of lysis buffer and stored at -20°C until further use.

Purification

Procedure
Column 1: Ni-affinity, HisTrap, 1 ml (GE/Amersham Biosciences)
Column 2: Hiload 16/60 Superdex 200 prep grade 120 ml (GE/Amersham Biosciences)

AKTA Xpress Affinity/Gel Filtration. 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 A280nm was automatically collected.
AKTA Xpress Affinity/Gel Filtration. The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column in GF buffer at 0.80 ml/min. Eluted protein was collected in 2 ml fractions in a 96well block.

Extraction

Procedure
The resuspended pellet was thawed and homogenised by using Emulsiflex-C5 homogenizer (Avestin) 5x and then centrifuged at 4°C in Beckman JA-17 rotor at 16000 rpm for 45 min. The AKTAxpress system was used.
Concentration: The protein was concentrated to 9.1mg/ml by using an Amicon Ultra 10k concentrator (Millipore).
Ligand
MassSpec: The experimentally determined mass of ACADVLA was 65729Da, which corresponds to the theoretical mass.
Crystallization: Prior to crystallization, FAD was added to a final concentration of 10 μM. Crystals were grown by vapour diffusion in nanolitre sitting drops at 4°C. Nanodrops comprising 50nl protein solution (9.1mg/ml) and 100nl reservoir solution were equilibrated against reservoir solution (15% PEG3350, 0.1M sodium succinate pH7.0). Prior to data collection, crystals were flash frozen from mother liquor supplemented with 25% ethylene glycol.
NMR Spectroscopy:
Data Collection: Resolution: 1.45 Å. X-ray source: Swiss Light Source, beamline SLS-X10SA, single wavelength.
Data Processing: