AK5

PDB:2BWJ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_777283
Entry Clone Source:TCK
SGC Clone Accession:
Tag:TEV-cleavable (*) N-terminal his6 tag: MHHHHHHSSGVDLGTENLYFQ*S(M).
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

```
mhhhhhhssgdlgtenlyfqsMGGFMEDLRKCKIIFIIGGPGSGKGTQCEKLVEKYGFTHLSTGELLREELASESERSKLIRDIMER
GDLVPSGIVLELLKEAMVASLGDTRGFLIDGYPREVKQGEEFGRRIGDPQLVICMDCSADTMTNRLLQRSRSSLPVDDTTKTIAKRL
EAYYRASIPVIAYYETKTQLHKINAEGTPEDVFLQLCTAIDSIflMutation R135M
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Vector:pLIC- SGC1

Growth

Medium:
Antibiotics:
Procedure: Grow starter cultures from freshly transformed colonies in 10 mL LB, 0.1 mg/mL ampiciline. This started culture was diluted 1:1000 in fresh media and was grown at 37°C to an OD600 of 0.4 and than transferred to 18°C. Expression was induced at an OD600 of 0.6 - 0.7 using 1 mM IPTG (final concentration). Cells were harvested after 4h by centrifugation (15min, 6000rpm on a JLA 8.100 rotor), transferred to 50-mL tubes, and frozen at -20°C.

Purification

Procedure
Column 1 : A DE52 column (10 g in 100 mL of 2.5 M NaCl) was equilibrated with 100mL of Loading Buffer. A 5 mL NiNTA column was equilibrated with 20mL of Loading Buffer.

Buffers: Loading buffer: 50 mM Hepes, pH 7.5, 500 mM NaCl, 5% glycerol. Wash buffer: 50 mM Hepes, pH 7.5, 500 mM NaCl, 20 mM imidazole, 5% glycerol. Elution buffer: 50 mM Hepes, pH 7.5, 500 mM NaCl, 50-250 mM imidazole (step elution), 5% glycerol.

Procedure: A DE52 column (10gr suspended in 100mL of 2.5M NaCl) was equilibrated with 100mL of Loading Buffer. A 5mL NiNTA column was equilibrated with 20mL of loading buffer. The lysed sample was applied to the DE-52 column and washed through with 50 mL loading buffer. The flow through was applied to the 5 mL Ni-NTA column which was washed with 2x10mL of wash buffer and eluted with elution buffer in 5 mL aliquots (Step elution using 50, 100, 150, 200 and 250 mM imidazole in the Elution Buffer)

Column 2: SEC

Buffers : Gel Filtration Buffer: 10mM HEPES, pH 7.5, 100mM NaCl

Procedure : AKTA-prime. Fractions containing AK5A collected from IMAC and treated with TEV protease overnight (identified by SDS PAGE) were concentrated to about 1.5mL and directly applied to a S75 16/60 column equilibrated in 10 mM Hepes pH 7.5, 100 m NaCl. The flow rate was 1mL/min and the pure protein eluted at 60-70min.

Enzymatic treatment: Treated the IMAC elution(s) with TEV protease overnight at 4°C.

Extraction

Procedure
Extraction buffer: 50 mM HEPES, pH 7.5, 500 mM NaCl, 5% Glycerol.

The cell pellets (5 g wet wt) were re-suspended in 50 mL extraction buffer containing a Protease Inhibitor cocktail tablet (Roche), and lysed in a high pressure cell disrupter. The supernatant was centrifuged for 30 minutes at 35k g in a JA 25.5 rotor at 4°C.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were grown at 4°C in 600 nL sitting drops mixing 300 nL of AK5A (15 mg/mL in 10mM Hepes pH 7.5, 100mM NaCl ,10mM DTT) with 300nl of a solution containing 0.1M NaCl, 0.1M HEPES pH 7.5 and 1.6M (NH4)2SO4. Adenosine monophosphate (AMP), Adenylyl imidodiphosphate (AMPPNP) and Mg+2 were also present in the crystallization matrix at a final concentration of 1mM each. Cryo protection was achieved by adding to the crystallization mix sucrose (25% final w/v).
NMR Spectroscopy:
Data Collection:
Data Processing: