CAPN1

PDB:2ARY

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:12408656
Entry Clone Source:MGC
SGC Clone Accession:capn01.029.360:A5; SDC002 A5
Tag:N-terminal histag with integrated thrombin cleavage site: mgsshhhhhhssglvpr*gs
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsGRHENAIKYLGQDYEQLRVRCLQSGTLFRDEAFPPVPQSLGYKDLGPNSSKTYGIKWKRPTELLSNPQ
FIVDGATRTDICQGALGDCWLLAAIASLTLNDTLLHRVVPHGQSFQNGYAGIFHFQLWQFGEWVDVVVDDLLPIKDGKLVFVHSAEG
NEFWSALLEKAYAKVNGSYEALSGGSTSEGFEDFTGGVTEWYELRKAPSDLYQIILKALERGSLLGCSIDISSVLDMEAITFKKLVK
GHAYSVTGAKQVNYRGQVVSLIRMRNPWGEVEWTGAWSDSSSEWNNVDPYERDQLRVKMEDGEFWMSFRDFMREFTRLEICNLTPDA
LKS

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure: Using the SGC's LEX bubbling system, CAPN1 was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 µg/mL of kanamycin at 37ºC to an OD600 of 7.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15ºC. The culture was centrifuged and the cell pellets were collected and stored at -80ºC.

Purification

Procedure
IMAC purification: 4 microL of clarified supernatant is reserved for later analysis by SDS-PAGE. The rest of the clarified supernatant is then diluted 1:2 in lysis buffer, and loaded at approximately 1mL/min by gravity onto 5 mL of Ni-NTA resin (Qiagen 30450). 5 column volumes of lysis buffer are used to wash the column at approximately 3 mL/min, followed by 5 column volumes of low imidazole buffer (lysis buffer + 10 mM Imidazole (VWR EM-5720) pH 8) at approximately 3 mL/min. A 4 µL sample of the low imidazole wash is saved for later

analysis by SDS-PAGE. Samples are eluted from the Ni-NTA resin by exposure to 10 mL elution buffer (lysis buffer + 250 mM imidazole and 10% glycerol (EMD GX0185-5)) at 1mL/min flow rate. A 10 µL sample of the eluate is saved for SDS-PAGE analysis. 10 µL of each eluate is saved for measurement of protein concentration using Bradford reagent (BioRad 500-0202).

Size exclusion chromatography: An XK 16x65 column (part numbers 18-1031-47 and 18-6488-01, GE Healthcare) packed with HighLoad Superdex 200 resin (10-1043-04, GE Healthcare) is pre-equilibrated with gel filtration buffer (lysis buffer + 5 mM ß-ME (Sigma 63689)) for 1.5 column volumes using an AKTAxpress (18-6645-05, GE Healthcare) at a flow rate of 3 mL/min. 5 mL of sample is loaded onto the column at 1.5 mL/min, and 2mL fractions are collected into 96-well plates (VWR 40002-012) using peak fractionation protocols with the following parameters: (Slope; min. peak width 0.833 min; level 0.000 mAU; peak start slope 10.000 AU/min; peak end slope 20.000 AU/min). Peak fractions are analyzed for purity using SDS-PAGE or visual analysis of the chromatogram and pooled.

Protein concentration: Purified proteins are concentrated using either 4 mL or 15 mL concentrators with an appropriate molecular weight cut-off (Amicon Ultra-15 10,000 MWCO, UFC901024 or 5,000 MWCO, UFC900524, as appropriate, Millipore) . The protein is first concentrated, then diluted 10-fold into low salt crystallization buffer (50 mM Tris pH 8.0, 100 mM NaCl, 1mM EDTA, 2 mM $CaCl_2$) to a final concentration of 20 mg/mL for crystallographic screening or other biophysical studies.

Extraction

Procedure
Frozen cell pellets contained in bags (Beckman 369256) obtained from 2L liters of culture are thawed by soaking in warm water for 5 minutes. Each cell pellet is resuspended in 20 mL lysis buffer (50 mM Tris pH 8.0 (VWR EM-9210), 500 mM NaCl (VWR EM-SX0420-1), 1mM EDTA), 1mM phenylmethanesulfonyl fluoride (Sigma P7626), 2mM CaCl2 and 1mL Sigma general protease inhibitor (Sigma P2714-1BTL, resuspended according to manufacturerÂ□s instructions) and then homogenized using an Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60 seconds per pellet. Cell lysis is accomplished by sonication (Virtis408912, Virsonic) on ice: the sonication protocol is 10 sec pulse at half-maximal frequency (5.0), 10 second rest, for 6 minutes total sonication time per pellet. Lysed cells are placed into centrifuge tubes (363647, Beckman Coulter) and centrifuged in a JA25.50 rotor in an Avanti J-20 XPI centrifuge (Beckman Coulter) for 20 minutes at 69,673 x g. The supernatant is decanted into a beaker, and the insoluble pellet discarded.
Concentration:
Ligand
MassSpec:
Crystallization: Purified CAPN1 was crystallized using the hanging drop vapor diffusion method. Diffracting crystals leading to the structure grew when the protein was mixed with the reservoir solution (containing 1.5 M ammonium formate, 0.1 M tris, pH 7.75) in a 1:1 volume ratio.
NMR Spectroscopy:
Data Collection:
Data Processing: