SYN3: Human synapsin III in complex with AMPPNP

PDB:2P0A

Revision

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Entry Clone Accession:IMAGE:30915375; gi|19924105
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SGC Clone Accession:SYN3A-c006
Tag:N-terminal Hexahistidine tag followed by a TEV cleavage site
Host:BL-21(DE3)R3 phage resistant

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*smGLMEP PGPSTPIVQRPRILLVIDDAHTDWSKYFH GKKVNGEIEIRVEQAEFSELNLAAYVT
GG CMVDMQVVRNGTKVVSRSFKPDFILVRQH AYSMALGEDYRSLVIGLQYGGLPAVNSLY SVYNFCSKPWVFSQLIKIFHSLGP
EKFPL VEQTFFPNHKPMVTAPHFPVVVKLGHAHA GMGKIKVENQLDFQDITSVVAMAKTYATT EAFIDSKYDIRIQKIGSNYKA
YMRTSISG NWKANTGSAMLEQVAMTERYRLWVDSCSE MFGGLDICAVKAVHSKDGRDYIIEVMDSS MPLIGEHVEEDRQLMADL
VVSKMSQLPMP GGTAPSPLRPWAPQIKSA

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Freshly transformed BL-21(DE3) phage resistant cells were used to innoculate 50 mls of LB + 50 microG/mL kanamycin and grown at 37degC overnight as a starter culture for a 1 litre growth. The large scale growth was grown at 37degC until approximately 30 mins before induction (OD600= 3) when the temperature was lowered to 25degC. After 1 hr protein production was induced with the addition of 0.5 mM IPTG. The next day cells were harvested by centrifugation at 6500 rpm for 15 minutes and resuspended in Lysis buffer before storage in the -80degC freezer.

Purification

Procedure
Ni-affinity: The cell extract (supernatant) was loaded on the column containing 1.5 ml bed volume of Ni-NTA (Qiagen). The column was then washed with 15 column volumes of Binding Buffer to equilibriate the resin, 40 column volumes of Binding Buffer and 20 column volumes of

Wash Buffer. SYN3 protein was eluted with 10 column volumes of Elution Buffer and collected in 2ml fractions.

Enzymatic treatment : At this stage the purity of the protein was greater than 95 % based on SDS-PAGE analysis. The C-terminal hexahistidine tag was removed by TEV protease treatment. The TEV protease, a hexahistidine-tagged construct, was over-expressed and purified in-house to a final concentration of 2.5 mg/ml.
Add 250 microL of the TEV protease was added to the pooled fractions and left at 4degC overnight. The following steps were carried out to remove the cleaved products and TEV protease.

Place 200 µl of 50 % Ni-NTA agarose in a 1.5 ml gravity column, add 1ml of 50mM HEPES pH 7.4, 500mM NaCl, 0.5mM TCEP to wash the resin (GF buffer) . Resuspend in GF buffer and add the TEV treated protein sample to the resin for batch binding over 60 min at 4°C . Finally reform the column and collect the supernatant which contains the cleaved SYN3A domain. The supernatant was filtered through a 0.45 µm syringe filter.

The TEV treated sample was loaded on the gel filtration column in GF buffer at 1.0 ml/min. Eluted proteins were collected in 1 ml fractions. The fractions containing SYN3A were pooled and treated overnight with 200 µl TEV protease. Complete tag cleavage was determined by Mass Spec before concentration of the fractions into a final volume of 2 ml. The sample was further clarified by filtration with a 0.2 µm Milipore syringe filter.

Column 3: 1.5ml Ni-affinity Qiagen gravity column.

Gel filtration: Any remaining contaminants and uncleaved products were removed by Ni-NTA batch binding (resin cleaning procedure as outlined above) with 1.5ml Ni-affinity Qiagen for 1 hour before collecting the eluatant (cleaved pure protein) which passes through the gravity column.

Concentration: The sample was then concentrated to 2 mg/ml using a 5 kD MW cutoff spin concentrator before storage in a -80°C freezer.

Extraction

Procedure
PMSF (final concentration 10 mM) in 10 ml of Lysis/ Binding Buffer was added to the thawed cell pellet (approximately 35 mL volume) and mixed by inversion. The cell pellet was lysed by passing it four times through the EmulsiFlex C5 high pressure homogeniser, collecting a final volume of approximately 2x 40 mL. PEI was added to a final concentration of 0.15%, mixed by inversion and the cell debris and precipitated DNA were spun down at 21,500rpm for 45 mins).
Concentration:
Ligand
MassSpec:EXPECTED MWt: 38372; MEASURED MWt: 38372.2
Crystallization: Before crystallisation AMP-PNP (adenosine 5'-(bg imido)triphosphate tetralithium salt hydrate) was added directly to SYN3A (1 µl of a 100mM stock added to 4 µl of concentrated protein) . Crystals grew from a 2:1 ratio mix of SYN3A-to-reservoir (1.26 M (NH4)2SO4; 0.2M Li2SO4; 0.1M TRIS pH 8.5) at 20°C. Crystal form was rods. Cryoprotection 20% Ethylene glycol.
NMR Spectroscopy:
Data Collection: Resolution: 1.9Å; X-ray source: Synchrotron SLS-X10, single wavelenght.
Data Processing: