CAMK2D

PDB:2VN9

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:IMAGE:5178265
Entry Clone Source:MGC
SGC Clone Accession:
Tag:*Cleavable N-terminal His6 tag.
Host:BL21 (DE3) Rosetta-Phage resistant

Construct

Prelude:
Sequence:

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lowercase refers to tag sequencemhhhhhhssgvdlgtenlyfq*smTDEYQLFEELGKGAFSVVRRCMKIPTGQEYA
AKIINTKKLSARDHQKLEREARICRLLKHPNIVRLHDSISEEGFHYLVFDLVTGGELFEDIVAREYYSEADASHCIQQILESVNHCH
LNGIVHRDLKPENLLLASKSKGAAVKLADFGLAIEVQGDQQAWFGFAGTPGYLSPEVLRKDPYGKPVDMWACGVILYILLVGYPPFW
DEDQHRLYQQIKAGAYDFPSPEWDTVTPEAKDLINKMLTINPAKRITASEALKHPWICQRSTVASMMHRQETVDCLKKFNARRKLKG
AILTTMLATRNFSAAKSLLKKPDGVKESTESSN
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Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Transformed 50 µl competent BL-21 (DE3) phage resistant cells with 10 µl of the plasmid DNA and plated out onto LB plate plus 50 µg/ml kanamycin and 35 µg/ml chloramphenicol. The next day colonies were picked into fresh deep well blocks containing 1 ml LB + 50 µg/ml kanamycin and 35 µg/ml chloramphenicol. Cultures were grown overnight and glycerol stocks were prepared by adding 333 ml of 60 % glycerol to 1 ml of cell suspension, which were stored at -80°C and used for future scale up preparations.The glycerol stock was used to inoculate 10 ml of LB supplemented with 50 µg/ml kanamycin and 35µg/ml chloramphenicol. This starter culture was grown overnight at 37°C and used to inoculate a 1 liter culture in the same medium. The culture was grown at 37°C until the OD600reached ~0.5. After that the temperature was lowered to 18°C. Protein production was induced with 1mM IPTG and recombinant CAMK2D was expressed at that temperature over night. The next day cells were harvested by centrifugation at 4000 rpm for 15 minutes. The cell pellet was stored at -80°C degrees.

Purification

Procedure
Lysis and Ni-affinity chromatography: Buffers: Binding buffer: 50 mM HEPES pH 7.5, 300mM NaCl,, 20 mM Imidazole. Wash buffer 1: 50 mM HEPES pH 7.5, 1M NaCl, 20mM Imidazole. Wash buffer 2:as for lysis buffer. Elution buffer: 50mM HEPES pH 7.5, 300mM NaCl, 150 mM Imidazole.Procedure: The cell pellet (38 g) was re-suspended in one volume (38 ml) of binding buffer. The re-suspended cells were lysed by sonication. The lysate was cleared by a centrifugation at 20,000 rpm (4°C).5 ml of 50% Ni-NTA slurry (Qiagen) was applied to a 1.5 x 10 cm gravity column. The column was equilibrated with 100 ml binding buffer. The lysate was applied to the column and was subsequently washed with 50 ml wash buffer 1 and 2. CAMK2D was eluted with 25 mls of elution buffer. The eluted protein was collected and analyzed by SDS-PAGE. DTT was added to the protein sample to a final concentration of 10mM. The protein was dephosphorylated with l-phosphatase (and the addition of MnCl2 to 50 mM). The N-terminal his6-tag was cleaved by incubating the protein overnight with TEV protease.Column 2: Size exclusion chromatography (Superdex S75, 60 x 1cm)SEC-Buffers: 50 mM Hepes, pH 7.5, 300 mM NaCl, 5 mM DTT. The fractions eluted of the Ni-affinity chromatography were concentrated to about 4 mls using Centricon concentrators (10kDa cut off). The concentrated protein was applied to a Superdex S75 column equilibrated in SEC buffer at a flow rate of 0.8 ml/min. Eluted fractions were 95% pure as judged by SDS-PAGE.

Extraction

Procedure

Concentration:10.6 mg/ml in SEC buffer using a centricon with a 10kDa cut off
Ligand
ASC65 (kindly provided by Prof. Kevin Shokat, University of California San Francisco)**MassSpec:**ESI-MS revealed that the protein had the expected mass of 36953 Da for the TEV cleaved protein
Crystallization:CAMK2D was crystallized at 4°C using the sitting-drop vapor diffusion method at 11.6 mg/ml in the presence of 1 mM ASC65 (kindly provided by Prof. Kevin Shokat, University of California San Francisco) added from a 50 mM DMSO stock. Diffraction quality crystals were obtained by mixing 100 nl of protein solution with 50 nl of 1.60M Na/KPO4; 0.1M HEPES pH 7.5.
NMR Spectroscopy:
Data Collection: Crystals were flash frozen in liquid nitrogen after being cryo protected in 30% sucrose (w/v) in the well solution. Diffraction data were collected to 2.1 Å at the Swiss light source beam-line X10SA at a single wavelength of 0.9999 Å.
Data Processing: