DAPK3 + K01742a

PDB:3BHY

Revision

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Entry Clone Accession:NM_001348
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Tag:N-terminal his6 tag with TEV cleavage site.
Host:BL21 (DE3) R3 (Phage resistant strain) cotransformed with a chloramphenicol resistant co-expression plasmid (pCOEX) expressing l-phosphatase.

Construct

Prelude:
Sequence:

TEV cleavage site indicated by ^, non-DAPK3 sequence indicated by lower casemhhhhhhssgv
dlgtenlyfq^smVEDHYEMGEELGSGQFAIVRKCRQKGTGKEYAAKFIKKRRLSSSRRGVSREEIEREVNILREIRHPNIITLHDI
FENKTDVVLILELVSGGELFDFLAEKESLTEDEATQFLKQILDGVHYLHSKRIAHFDLKPENIMLLDKNVPNPRIKLIDFGIAHKIE
AGNEFKNIFGTPEFVAPEIVNYEPLGLEADMWSIGVITYILLSGASPFLGETKQETLTNISAVNYDFDEEYFSNTSELAKDFIRRLL
VKDPKRRMTIAQSLEHSWIKAIRRRNVRGEDSG

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:1ml from a 20 ml overnight was used to inoculate 2 l of TB medium containing 50µg/ml Kanamycine and 40µg/ml chloramphenicol. E .coli cells were grown in 2.5-L baffled flasks at 37 degrees until OD reached 2.0. The cells were cooled to 25°C and expression of DAPK3 was induced adding 0.5 mM IPTG at an OD600 of 2.2.

Purification

Procedure
Column 1: : Ni-affinity, HisTrap, 5 ml (GE/Amersham Biosciences) Buffer: Lysis buffer: 50 mM HEPES buffer, pH 7.5, 500 mM NaCl, 5% glycerol; Wash buffer: 50 mM HEPES buffer, pH 7.5, 500 mM NaCl, 20 mM imidazole, 5% Glycerol; Elution buffer: 50 mM HEPES buffer, pH 7.5, 500 mM NaCl, 150 mM imidazole, 5% Glycerol.Procedure: The cell extract was loaded on the column under gravity. The column was then washed with 10 volumes of lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer. DTT was added to 5 mM and the His-tag was

removed with the addition of approximately 200µg of Tev protease. The protein was incubated at 4°C for about 14 hours. The completion of Tev digestion was monitored by ESI-mass spectrometric analysis. Further Tev was added if necessary and the protein was further incubated at 4°C as required.Column 2: Gelfiltration, Hiload 16/60 Superdex 200 prep grade, 120 ml (GE/ Amersham Bioscience Buffers: 50 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 5 mM DTT Procedure: The Tev digested protein was concentrated by using an Amicon Ultra ultrafiltration unit (10K cutoff) and was loaded on the gel filtration column at 0.80 ml/min. Eluted proteins were collected in 1.8 ml fractions.

Extraction

Procedure
Lysis buffer: 50 mM HEPES buffer, pH 7.5, 500 mM NaCl, 5% Glycerol.35 ml of frozen cell pellets (2l culture) were thawed at room temperature. The cells were lysed by sonication for a total of 3 minutes (20 seconds on, 50 seconds off at 40% maximum frequency) on ice. Nucleic acids and cell debris were removed by centrifugation for 45 minutes at 30000xg.
Concentration: The diluted purified samples were concentrated by using an Amicon Ultra ultrafiltration unit (10K cutoff) to 12.6 mg/ml. The protein concentration was determined spectrophotometrically using the calculated molar extinction coefficient.
Ligand
beta-carboline inhibitor (kindly provided by the laboratory of Prof Bracher, University of Munich)**MassSpec:**The mass of the recombinant protein was in agreement with the mass calculated from the expected sequence.
Crystallization:(3BHY): Crystals of unphosphorylated DAPK3 in the presence of a beta-carboline inhibitor (kindly provided by the laboratory of Prof Bracher, University of Munich) were grown in 0.1M SPG pH 6.0; 30.0% PEG 1K. The crystal was directly frozen in the crystallization solution.
NMR Spectroscopy:
Data Collection:(3BHY): 1.25 Å diffraction data (space group P21) were collected for unphosphorytlated DAPK3 in complex with beta carboline inhibitor at the Swiss light source (SLS) X10SA at a single wavelength of 0.9807 Å.
Data Processing: