SLK di-phosphorylated + K00546

PDB:2JFL

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_055535.2
Entry Clone Source:synthetic DNA
SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmKQYEHV KRDLNPEDFWEIIGELGDGAFGKVYKAQN KETSVLAAAKVIDTKSEEELEDYMVEI
DI LASCDHPNIVKLLDAFYYENNLWILIEFC AGGAVDAVMLELERPLTESQIQVVCKQTL DALNYLHDNKIIHRDLKAGNILFT
LDGDI KLADFGVSAKNTRTIQRRDSFIGTPYWMA PEVVMCETSKDRPYDYKADVWSLGITLIE MAEIEPPHHELNPMRVLLKIA
KSEPPTLA QPSRWSSNFKDFLKKCLEKNVDARWTTSQ LLQHPFVTVDSNKPIRELIAEAKAEVTEE VEDGKE

Vector:

Growth

Medium:
Antibiotics:
Procedure:1ml from a 10 ml overnight culture (BL21 Rosetta strain) containing 50 µg/ml kanamycine and 35 µg/ml chloramphenicole was used to inoculate 1 liter of LB media containing the same concentration of antibiotics. Cultures were grown at 37°C until the OD600 reached ~0.3. After that the temperature was adjusted to 20°C. Expression was induced for 4 hours using 1mM IPTG at an OD600 of 0.8. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen.

Purification

Procedure
Column 1: Ni-affinity chromatography
5 ml of 50% Ni-NTA slurry (Qiagen) was applied to a 1.5 x 10 cm gravity column. The column was equilibrated with 50 ml binding buffer. The lysate was applied to the column which was subsequently washed with 50 ml wash buffer 1 and 2. SLK 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 N-terminal his 6 -tag was cleaved by incubating the protein overnight with TEV protease. Column 2: Size exclusion chromatography (Superdex S75, 60 x 1cm)
Procedure: 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.

Concentration: Centricon with a 10kDa cut off in SEC-buffer.

Extraction

Procedure
pellets were lysed by sonication. The lysate was centrifuged at 19,000 rpm for 60 minutes and the supernatant collected for purification.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained using the vapor diffusion method and a protein concentration of 10 mg/ml containing 1 mM Cdk1/2 Inhibitor III (CalbioChem) by mixing 100nl of the concentrated protein with 50nl of a well solution containing 16% PEG3350, 0.15M KSCN, 0.1M BisTris propane pH 6.5 and 10 % ethylene glycol. Crystals of the 4-(4- (5-cyclopropyl- 1h-pyrazol-3- ylamino)- quinazolin-2- ylamino)- benzonitrile complex were obtained using essentially mono-phosphorylated protein a protein concentration of 10 mg/ml containing 1 mM of the inhibitor by mixing 100 nanoL of protein solution with 50 nl of a buffer containing 18% PEG3350, 0.15M KSCN, 0.1M BisTris propane pH 6.5 and 10 % ethylene glycol. In order to obtain crystals of phosphorylated apo-SLK and the di-phosphorylated complex with 4-(4- (5-cyclopropyl- 1h-pyrazol-3- ylamino)- quinazolin-2- ylamino)- benzonitrile the recombinant protein was incubated with 5 mM ATP/Mg overnight. Apo-SLK crystallized using 0.20M KSCN; 0.1M BisTris propane pH 7.5; 20.0% PEG 3350; 10.0% ethylene glycol. Crystals appeared after a couple of days at 4degC.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected using the well solution supplemented with an additional 15% ethylene glycol and flash frozen in liquid nitrogen. All diffraction data were collected at the SLS beam line X10 (λ=0.979 Å).
Data Processing: