SOCS6 + phosphopeptide

PDB:2VIF

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi| 21450784
Entry Clone Source: FivePrime
SGC Clone Accession:
Tag: Cleavable N-terminal His6 tag (*): mhhhhhhssgvdlgtenlyfq*SM
Host: Phage-resistant derivative of BL21(DE3)

Construct

Prelude:
Sequence:

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mhhhhhhssgvdlgtenlyfq*SMVQSSGPM-D-VTSLTEELKKLAKQGWYWGPITRWEAEGKLANVPDGSFLVRDSSDDRYLLSLS
FRSHGKTLHTRIEHSNGRFSFYEQPDVEGHTSIVDLIEHSI-G-DSENGAFCYSRSRLPGSATYPVRLTNPVSRFMQVRS

Two amino acids changes from gi| 21450784 originated from PCR and are highlighted.
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Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:5ml from a 50 ml overnight TB culture containing 50 µg/ml kanamycin/34 µg/ml chloramphenicol was used to inoculate each of 4x 1 litre of TB containing 50 µg/ml kanamycin/34 µg/ml chloramphenicol. Cultures were grown at 37°C until the OD600 reached ~2.0 then the temperature was adjusted to 18°C. Expression was induced overnight using 0.5 mM IPTG. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen. Binding buffer: 50mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerol.

Purification

Procedure
Column 1: Ion exchange - Nucleic acid removal. DEAE cellulose (DE52, Whatman), 10 g of resin in 2.5 x 20 cm column. The resin was hydrated in 2.5M NaCl, washed with 20 ml binding buffer prior to loading the sample.Buffers: 50mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerolProcedure: Supernatant was applied by gravity flow, followed by a wash with 50 ml binding buffer. The column flow-through was collected.Column 2: Ni-affinity. Ni-sepharose (Amersham), 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.Buffers: Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM imidazole, 5% glycerol; Wash buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM Imidazole, 5% glycerol; Elution buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 50 to 250 mM Imidazole, 5% GlycerolProcedure: The flowthrough from column 1 was loaded by gravity flow on the Ni-sepharose column. The column was then washed with 100 ml wash buffer under gravity flow. The protein was eluted by applying 5-ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150 mM and 250 mM); fractions were collected until essentially all protein was eluted. 50 mM L-arginine/50 mM L-glutamate and 10 mM DTT were added for overnight storage together with TEV protease for cleavage of the N-terminal hexahistidine tag.Column 3: Size Exclusion Chromatography. Superdex S75 16/60 HiLoadBuffers : 50 mM HEPES pH 7.5, 150 mM NaCl, 5 mM DTTProcedure: The TEV-cleaved protein was concentrated to 3ml using an Amicon 5 kDa cut-off concentrator and ran on a S75 gel filtration column. Elution fractions containing SOCS6 were pooled and an additional 5 mM DTT was added for overnight storage.Column 4: Ion Exchange Chromatography. Source15QBuffers: Buffer A: 50 mM HEPES pH 7.5; Buffer B: 50 mM HEPES pH 7.5, 2M NaClProcedure: The gel filtration elution was diluted five fold with BufferA and loaded onto a Source15Q anion exchange column equilibrated in the same buffer. SOCS6 did not bind the column and was collected in the flow through. Contaminants bound the column and were eluted with BufferB.

Extraction

Procedure
Extraction method: Frozen pellets were thawed and fresh 1 mM PMSF and 0.5 mM TCEP were added. Cells were lysed by sonication. The lysate was centrifuged at 16,000 rpm for 60 minutes and the supernatant collected for purification.
Concentration: Protein concentration: The protein buffer was adjusted to 25 mM HEPES pH 7.5, 100 mM NaCl, 10 mM DTT, 50 mM L-arginine/50 mM L-glutamate and concentrated to 13.0 mg/ml using an Amicon 5 kDa cut-off concentrator. An excess of phosphopeptide (NGNN(pY)VYIDPT derived from c-KIT pY568) was present during concentration and a further 2mM peptide was added from dry powder to the final crystal sample.
Ligand
phosphopeptide (NGNN(pY)VYIDPT derived from c-KIT pY568)**MassSpec:**Mass spec characterization: LC- ESI -MS TOF. Expected mass (after TEV cleavage): 18363. The expected mass was observed.

Crystallization: Crystallization: Crystals were grown at 4°C in 150 nl sitting drops mixing 50 nl of protein with 100 nl of a solution containing 0.2M (NH4)2SO4; 0.1M MES pH 6.5; 30% mPEG 5K. The crystals were cryo-protected using 20% ethylene glycol which was added to the drop 20 seconds prior to mounting and flash freezing in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data collection: Resolution: 1.45 Å. X-ray source: Diffraction data were collected at the SLS beamline X10SA at a single wavelength (0.98248 Å).
Data Processing: