DYRK1A + K00611a

PDB:2VX3

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|18765758
Entry Clone Source:synthetic
SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

```
mhhhhhhssgvdlgtenlyfq^smSSHKKERKVYNDGYDDDNYDYIVKNGEKWMDRYEIDSLIGKGSFGQVVKAYDRVEQEWVAIKI
IKNKKAFLNQAQIEVRLLELMNKHDTEMKYYIVHLKRHFMFRNHLCLVFEMLSYNLYDLLRNTNFRGVSLNLTRKFAQQMCTALLFL
ATPELSIIHCDLKPENILLCNPKRSAIKIVDFGSSCQLGQRIYQYIQSRFYRSPEVLLGMPYDLAIDMWSLGCILVEMHTGEPLFSG
ANEVDQMNKIVEVLGIPPAHILDQAPKARKFFEKLPDGTWNLKKTKDGKREYKPPGTRKLHNILGVETGGPGGRRAGESGHTVADYL
KFKDLILRMLDYDPKTRIQPYYALQHSFFKKTADE
```

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: 1ml from a 10 ml overnight culture containing 50 µg/ml kanamycin and 35 µg/ml chloramphenicol was used to inoculate 1 liter of LB media containing the same concentration of antibiotics. Cultures were grown at 37°C until the OD600reached ~0.3. After that the temperature was adjusted to 20°C. Expression was induced over night using 1mM IPTG at an OD600 of 0.6. 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% glycerol, 5 mM imidazole and 0.5mM TCEP.

Purification

Procedure
Column 1: Ni-affinity chromatography.Buffers: Binding buffer: Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl , 5% Glycerol, 5mM Imidazole. 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% Glycerol .Procedure: 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. The protein was eluted by gravity flow by applying 5 ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150mM, 250 mM); fractions were collected until essentially all protein was eluted. The eluted protein was analyzed by SDS-PAGE. DTT was added to the protein sample to a final concentration of 5mM.Column 2: Size exclusion chromatography (Superdex S200, 16/60)SEC-Buffers: 50 mM Hepes, pH 7.5, 500 mM NaCl, 5 mM DTT.Procedure: The fractions eluted of the Ni-affinity chromatography were concentrated to about 15 mls using Centricon concentrators (10kDa cut off). The concentrated protein was applied to a Superdex S200 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
Cell pellets were lysed by C5 high pressure homogenizer (Avestin). The lysate was centrifuged at 21,000 rpm for 60 minutes and the supernatant collected for purification.
Concentration: Centricon with a 30 kDa cut off in SEC-buffer.
Ligand
K00611a (an ATP mimetic inhibitor)**MassSpec:**The mass of the protein was calculated to be 44403 Da and experimentally determined mass was 40403.4 Da for the His tag containing protein. However, up to three phosphorylation sites very evident in the ESI-MS spectrum from which one had been identified to be localized at the activation segment tyrosine Y321. There was no evidence for additional sites in the electron density and we assume that two additional partially occupied sites are present in disordered regions of the protein that have not been included in this model. The identity of the protein was reconfirmed to be correct by DNA sequencing both DNA strands of this expression construct.
Crystallization: Crystals were obtained using the vapor diffusion method and a protein concentration of 14mg/ml by mixing 200nl of the concentrated protein with 100 nl of a well solution containing: 34w/v PEG 300; 0.1M LiSO4; 8.5 pH Tris.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected using the well solution supplemented and flash frozen in liquid nitrogen. Diffraction data were collected at the SLS beam line SAX10. Resolution: 2.5 Å.
Data Processing: