UCK1

PDB:2UVQ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|60551657
Entry Clone Source:
SGC Clone Accession:UCK1A-k005
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*sm
Host:*E.coli* BL21(DE3) gold transformed with the pRARE2 plasmid from Rosetta2

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmRPFLIGVSGGTASGKSTVCEKIMELLGQNEVEQRQRKVVILSQDRFYKVLTAEQKAKALKGQYN
FDHPDAFDNDLMHRTLKNIVEGKTVEVPTYDFVTHSRLPETTVVYPADVVLFEGILVFYSQEIRDMFHLRLFVDTDSDVRLSRRVLR
DVRRGRDLEQILTQYTTFVKPAFEEFCLPTKKYADVIIPRGVDNMVAINLIVQHIQDILNGDICKWHRGGS

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 50 ml LB supplemented with 50 µg/ml kanamycin at 37 ºC overnight. The overnight culture (20 ml) was used to inoculate 750 mll TB supplemented with 8 g/l glycerol and 50 µg/ml kanamycin. The culture was grown in TunAir flask at 37 ºC until OD600 reached ~2. The culture was down-tempered to 18 ºC over a period of 1 hour before target expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight and cells were harvested the following morning by centrifugation (4,400 x *g*, 10 min, 4 ºC). The resulting cell pellet (26 g wet cell weight) was stored at -80 ºC.

Purification

Procedure
Columns
IMAC: Ni-charged 1 ml HisTrap HP (GE Healthcare)
Gel filtration column: HiLoad 16/60 Superdex 200 Prep Grade (GE Healthcare)

Procedure
IMAC columns were equilibrated with IMAC wash1 buffer, and gel filtration columns were equilibrated with GF buffer. Purification of the protein was performed on an ÄKTAprime system (GE Healthcare). The filtered lysate was loaded onto the Ni-charged HiTrap Chelating column and washed with IMAC wash1 buffer followed by IMAC wash2 buffer. Bound protein was eluted from the IMAC column with IMAC elution buffer and loaded onto the gel filtration column. Fractions containing the target protein were identified by SDS-PAGE, pooled, and fresh TCEP was added to a final concentration of 2 mM. The protein was concentrated using a VIVASPIN 20 centrifugal filter device (10,000 NMWL; VWR) to 24 mg/ml in a volume of 0.45 ml. The identity of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The cell pellet was thawed and resuspended in Gel filtration buffer supplemented with 2000 U Benzonase (Merck) and one tablet of Complete EDTA-free protease inhibitor (Roche Applied Science). Cells were disrupted by sonication (Vibra-Cell, Sonics) at 80% amplitude for 3 min effective time (pulsed 4s on, 4s off) and cell debris was removed by centrifugation (49,000 x *g*, 30 min, 4 ºC). The supernatant was decanted and filtered through a 0.45 µm flask filter.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained by the hanging drop vapour diffusion method in a 24-well plate. 2 µl protein solution (10 and 15 mg/ml with 5 mM CMP added) was mixed with 1 µl of well solution consisting of 1 M succinic acid pH 6.7. The plate was incubated at 20 ºC and crystals appeared within a couple of days. The crystals were transferred to a cryo solution consisting of 1 M or 1.2 M succinic acid pH 7 complemented with 30% glycerol, 0.2 M NaCl and 10 mM ADP. The crystals were keept in the soak solution for 55 min and then flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data for the apo structure (2JEO) were collected at the ESRF, beamline ID14EH1 and processed using Mosflm and Scala of the CCP4 suite. The structure was solved to 2.5 Å with molecular replacement using Phaser and the human uridine-cytidine kinase 2 as template (1XRJ). Data for the ADP soaked crystals (2UVQ) were collected at BESSY, beamline BL14-1. The original structure was used for phasing the data set, which belonged to the same space group and was processed with the same programs to a resolution of 3 Å. The final models were refined with Refmac5.
Data Processing: