DDX52

PDB:3DKP

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC041785
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*sm
Host: *E.coli* BL21(DE3) R3 pRARE, where R3 denotes a derivative of BL21(DE3) resistant to a strain of T1 bacteriophage (SGC Oxford) and the pRARE plasmid originating from the Rosetta strain (Novagen) supplies tRNAs for rare codons.

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*smKINFLRNKHKIHVQGTDLPDPIATFQQLDQEYKINSRLLQNILDAGFQMPTPIQMQAIPVMLH
GRELLASAPTGSGKTLAFSIPILMQLKQPANKGFRALIISPTRELASQIHRELIKISEGTGFRIHMIHKAAVAAKKFGPKSSKKFDI
LVTTPNRLIYLLKQDPPGIDLASVEWLVVDESDKLFEDGKTGFRDQLASIFLACTSHKVRRAMFSATFAYDVEQWCKLNLDNVISVS
IGARNS

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 30 ml TB supplemented with 8 g/l glycerol, 100 µg/ml kanamycin and 34 µg/ml chloramphenicol at 30 ®C overnight. The overnight culture (20 ml) was used to inoculate 1.5 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and approximately 200 µl PPG P2,000 81380 anti-foam solution (Fluka). The culture was grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 ®C until OD600 reached ~2. The bottle was down-tempered to 18 ®C 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 ®C). The resulting cell pellet (33.2 g wet cell weight) was resuspended in lysis buffer (1.5 ml/g cell pellet), supplemented with 2500 U Benzonase (Merck) and 1 tablet of Complete EDTA-free protease inhibitor (Roche Applied Science). The cell suspension was stored at -80 ®C.

Purification

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

Procedure
Purification of the protein was performed as a two step process on an ÄKTAxpress system (GE Healthcare). Prior to purification, columns were equilibrated with IMAC wash1 buffer and gel filtration buffer, respectively. 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 automatically loaded onto the gel filtration column. Fractions containing the target protein were pooled and fresh TCEP was added to a final concentration of 2 mM. The protein was subsequently concentrated using an Amicon Ultra-15 centrifugal filter device with 10,000 NMWL (Millipore) to 49.9 mg/ml in a volume of 1.5 ml. ADP and MgCl2 was added to the concentrated protein to a final concentration of 5 mM and 10 mM respectively.

Tag removal
The N-terminal histidine tag was proteolytically removed by incubating the target protein with His-tagged TEV protease at a molar ratio of 30:1 at 4 ºC overnight. The proteolytic reaction went to completion, as judged by SDS-PAGE. Target protein was purified from tag and protease by passing the reaction mixture over a Ni-charged 1 ml HiTrap Chelating HP column (GE Healthcare) pre-equilibrated with IMAC wash1 buffer. The cleaved protein was concentrated and the buffer was changed to GF buffer using an Amicon Ultra-15 centrifugal filter device with 5,000 NMWL (Millipore). The final protein concentration was determined to 20.7 mg/ml in a volume of 0.46 ml. The identity of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The cell suspension was quickly thawed in water and diluted to 2 x 68 ml with lysis buffer. 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*, 20 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 sitting drop vapour diffusion method in a 96-well plate. 0.1 µl of the protein solution (diluted to 18.4 mg/ml) including 20 mM ADP and 10 mM MgCl2 was mixed with 0.2 µl of well solution consisting of 0.1 M imidazole, pH 8.0 and 10% (w/v) PEG 8000. The plate was incubated at 4 ºC and crystals appeared after two days. The crystal was quickly transferred to cryo solution consisting of 0.1 M imidazole, pH 8.0, 12% (w/v) PEG 800 and 30% glycerol, and flash-frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data to 2.1 Å resolution was collected from a single crystal at ESRF (ID23-1).
Data Processing: The crystal belonged to P21 space group with cell parameters of a=40.6 Å, b=38.4 Å, c=73.8 Å and a=90.0®, b=90.4®, c=90.0®. The structure was solved by molecular replacement using MOLREP with the Drosophila DEAD-box protein structure (2DB3) as a search model. The asymmetric unit consisted of one polypeptide chain. ADP and a Mg2+ ion were located at the active site. Structure was refined with REFMAC5 using 3 TLS groups for the

protein chain. Final R-values were: R=18.2% and Rfree=23.6%. Coordinates and structure factors were deposited in PDB with accession code 3DKP.