HSPA1L

PDB:3GDQ

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession:
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:HSPA1LA-k005
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*smATAKGIAIGIDLGTTYSCVGVFQHGKVEIIANDQGNRTTPSYVAFTDTERLIGDAAKNQVAMN
PQNTVFDAKRLIGRKFNDPVVQADMKLWPFQVINEGGKPKVLVSYKGENKAFYPEEISSMVLTKLKETAEAFLGHPVTNAVITVPAY
FNDSQRQATKDAGVIAGLNVLRIINEPTAAAIAYGLDKGGQGERHVLIFDLGGGTFDVSILTIDDGIFEVKATAGDTHLGGEDFDNR
LVSHFVEEFKRKHKKDISQNKRAVRRLRTACERAKRTLSSSTQANLEIDSLYEGIDFYTSITRARFEELCADLFRGTLEPVEKALRD
AKMDKAKIHDIVLVGGSTRIPKVQRLLQDYFNGRDLNKSINPDEAVAYGAAVQAAILMGDK

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from glycerol stock were plated onto a LB agar plate with antibiotics and incubated at 37 °C overnight. 10 colonies were used to inoculate 50 ml LB supplemented with 100 µg/ml kanamycin and 34 µg/ml chloramphenicol and grown at 37 °C overnight. 15 ml of the overnight culture were added to 1.5 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin, 34 µg/ml chloramphenicol and approximately 0.3 ml 204 Antifoam A6426 (Sigma). 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 (4,500 x *g*, 15 min, 4 °C). The resulting cell pellet (25.2 g wet cell weight) was resuspended in lysis buffer (2 ml/g cell pellet), supplemented with 0.5 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 ÄKTAprime system (GE Healthcare). Prior to purification, columns were equilibrated with IMAC wash1 buffer and gel filtration buffer, respectively. The filtered lysate was loaded onto two HiTrap Chelating columns connected in series and washed with IMAC wash1 buffer followed by IMAC wash2 buffer. Bound protein was eluted from the IMAC column with IMAC elution buffer and concentrated to 2 ml before loaded onto the gel filtration column. The target protein was eluted as two peaks from the gel filtration column. Fractions from the major peak (having a larger retention volume) were pooled and concentrated using an Amicon centrifugal filter device with 10,000 NMWL (Millipore) to 15.8 mg/ml in a volume of 1.75 ml.

Extraction

Procedure
The cell suspension was quickly thawed in water and 1000 U Benzonase (Merck) was added. 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 (49,100 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 using a 96-well plate. 0.2 µl of the protein sample (15.8 mg/ml) including 5 mM ADP and 5 mM MnCl2 was mixed with 0.1 µl of well solution consisting of 0.1 M Tris pH 8.5, 0.2 M trimethylamine n-oxide, 26% (w/v) PEG MME 2000. The plate was incubated at 4 ºC and crystals appeared within 2 days. For data collection crystals were quickly transferred to cryo solution consisting of well solution and 17% glycerol, and flash-frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data to 1.8 Å resolution was collected from a single crystal at DIAMOND (I03). The crystal belonged to space group P212121 with cell parameters a=70.1 Å, b=70.7 Å, c=97.5 Å, α= 90°, β=90°, γ= 90°.
Data Processing: The structure was solved by molecular replacement using MOLREP with the bovine heat-shock cognate 70 kDa protein (1BA0) as a search model. The asymmetric unit consisted of one polypeptide chain with one ADP, a phosphate ion and a Mn^{2+} ion located in the active site of the enzyme. The structure was refined with REFMAC5. Final R-values were R=17.22% and Rfree=20.25%. The coordinates and structure factors were deposited in the PDB with accession code 3GDQ.