HSPA2

PDB:3I33

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC001752
Entry Clone Source: Mammalian Gene Collection
SGC Clone Accession:HSPA2A-k002
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*sm
Host: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:

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mhhhhhhssgvdlgtenlyfq*smPAIGIDLGTTYSCVGVFQHGKVEIIANDQGNRTTPSYVAFTDTERLIGDAAKNQVAMNPTNTI
FDAKRLIGRKFEDATVQSDMKHWPFRVVSEGGKPKVQVEYKGETKTFFPEEISSMVLTKMKEIAEAYLGGKVHSAVITVPAYFNDSQ
RQATKDAGTITGLNVLRIINEPTAAAIAYGLDKKGCAGGEKNVLIFDLGGGTFDVSILTIEDGIFEVKSTAGDTHLGGEDFDNRMVS
HLAEEFKRKHKKDIGPNKRAVRRLRTACERAKRTLSSSTQASIEIDSLYEGVDFYTSITRARFEELNADLFRGTLEPVEKALRDAKL
DKGQIQEIVLVGGSTRIPKIQKLLQDFFNGKELNKSINPDEAVAYGAAVQAAILIGD
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Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 20 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 204 Antifoam A6426 (Sigma). The culture was grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 °C until OD600 reached ~2. The culture 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 (43 g wet cell weight) was resuspended in lysis buffer (1.5 ml/g cell pellet), supplemented with 2000 U Benzonase (Merck) and one tablet of Complete EDTA-free protease inhibitor (Roche Applied Science). The cell suspension was stored at -80 °C.

Purification

Procedure
Columns
IMAC: Ni-charged 2 ml HiTrap Chelating HP (GE Healthcare)
Gel filtration column: HiLoad 16/60 Superdex 75 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 ÄKTAxpress 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 automatically 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 an Amicon Ultra-15 centrifugal filter device (10,000 NMWL; Millipore) to 14 mg/ml in a volume of 0.31 ml. The identity of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The cell suspension was quickly thawed in water. 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 protein solution (14 mg/ml) including 5 mM ADP and 5 mM MgCl2 was mixed with 0.1 µl of well solution consisting of 0.1 M bis-Tris pH 5.5, 0.2 M ammonium acetate and 25% PEG 3350. The plate was incubated at 4 ºC. For data collection the crystals were quickly transferred to a cryo solution consisting of well solution complemented with 20% glycerol, and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data to 1.3 Å resolution was collected at ESRF (ID23-1), Grenoble, France
Data Processing: The structure was solved by molecular replacement using HSPA6 as template (PDB: 3FE1). The space group was P212121 with cell dimensions a=48.18 Å, b=78.6 Å, c=93.99 Å, α=β=γ=90°. The asymmetric unit consisted of one polypeptide chain with one ADP, a phosphate ion and a Mg2+ ion located in the active site of the enzyme. The structure was refined with REFMAC5. Data in the interval 25.56-1.30 Å resolution was used and at the end of the refinement the R values were: R=18.4% and Rfree=19.9%. Coordinates for the crystal structure were deposited in the Protein Data Bank, accession code 3I33.