DEADC1

PDB:3DH1

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC037955MGC
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:

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mhhhhhhssgvdlgtenlyfq*smEETEKWMEEAMHMAKEALENTEVPVGCLMVYNNEVVGKGRNEVNQTKNATRHAEMVAIDQVLD
WCRQSGKSPSEVFEHTVLYVTVEPCIMCAAALRLMKIPLVVYGCQNERFGGCGSVLNIASADLPNTGRPFQCIPGYRAEEAVEMLKT
FYKQENPNAPKSKVRK
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Vector:pNIC-BSA4

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 10 ml TB supplemented with 8 g/l glycerol, 100 µg/ml kanamycin and 34 µg/ml chloramphenicol at 30 °C overnight. The overnight culture (10 ml) was used to inoculate 0.75 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and approximately 100 µl Dow Corning anti-foam RD emulsion 63213 4D (BDH Silicone Products). 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 (22 g wet cell weight) was resuspended in lysis buffer (1 ml/g cell pellet), supplemented with 1000 U Benzonase (Merck) and 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 75 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 19.8 mg/ml in a volume of 0.75 ml. The identity of the protein was confirmed by mass spectrometry.

Extraction

Procedure
The cell suspension was quickly thawed in water and diluted to 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. 0.2 µl of the protein solution (19.8 mg/ml) was mixed with 0.1 µl of well solution consisting of 0.1 M bis-Tris pH 5.5, 0.2 M ammonium acetate, 25% (w/v) PEG 3350. The plate was incubated at 20 ºC and crystals appeared within 2 days. The crystal was quickly transferred to cryo solution consisting of 0.1 M bis-Tris pH 5.5, 0.2 M ammonium acetate, 26% PEG 3350 , 0.3 M NaCl and 20% glycerol, and flash-frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Data was collected at ESRF (ID23-1).
Data Processing: The crystal belonged to space group P21 with the cell parameters 47.53, 103.12, 77.59, 90.0, 97.6, 90.0,. Data was processed and scaled using XDS and XSCALE. The structure was solved by molecular replacement with MOLREP using the structure of tRNA-specific adenosine deaminase from *Streptococcus pyogenes* (PDB-code:2NX8) as search model. Simulated annealing was run in CNS, manual model building was done with COOT. The structure was refined with REFMAC5 using TLS in the end of the refinement. Data in the interval 20 - 2.8 Å resolution was used and final values for R and Rfree were 20.5% and 25.6% respectively. The coordinates for the crystal structure were deposited in the Protein Data Bank, accession code 3DH1.