ZADH1

PDB:2VNA

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NM_152444
Entry Clone Source: Origene
SGC Clone Accession:
Tag:C-terminal His-tag with TEV protease cleavage site (Tag sequence in lowercase below)
Host:E. coli strain: BL21(DE3)-R3 pRARE2 (previously known as Rosetta)

Construct

Prelude:
Sequence:

MMIVQRVVLNSRPGKNGNPVAENFRMEEVYLPDNINEGQVQVRTLYLSVDPYMRCRMNEDTGTDYITPWQLSQVVDGGGIGIIEESK
HTNLTKGDFVTSFYWPWQTKVILDGNSLEKVDPQLVDGHLSYFLGAIGMPGLTSLIGIQEKGHITAGSNKTMVVSGAAGACGSVAGQ
IGHFLGCSRVVGICGTHEKCILLTSELGFDAAINYKKDNVAEQLRESCPAGVDVYFDNVGGNISDTVISQMNENSHIILCGQISQYN
KDVPYPPPLSPAIEAIQKERNITRERFLVLNYKDKFEPGILQLSQWFKEGKLKIKETVINGLENMGAAFQSMMTGGNIGKQIVCISE
EIAENLYFQshhhhhhdykddddk

Vector: pNIC-CTHF

Growth

Medium:
Antibiotics:
Procedure:10 µl of a glycerol stock was inoculated into 5ml of TB medium (supplemented with 50µg/ml Kanamycin, 34µg/ml Chloramphenicol) and cultured at 37°C o/n in a shaking incubator (275 rpm). Next day 0.75 ml of o/n culture was used to inoculate 1 litre of TB medium (6x) and grown at 37°C with vigorous shaking (160 rpm) until the culture reaches an OD600 of 1.4. Temperature was reduced to 18°C, and cells were induced with IPTG at a concentration of 0.5 mM, and further cultivated for 16 hrs. Cells were harvested by centrifugation at 6500 rpm for 10 min, and the cell pellet was stored at -20°C until further use.

Purification

Procedure

Column 1: Ni-Sepharose 6 Fast Flow

Buffers: Lysis buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole; Wash buffer: 50 mM Tris-HCl pH 7.5, 500 mM NaCl, 5% Glycerol, 30 mM Imidazole; Elution buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole. note: All the buffers contain 0.5mM TCEP.

Procedure: The column was packed with 2 ml of Ni-Sepharose 6 Fast Flow slurry and equilibrated with 15 ml of binding buffer. The supernatant was loaded onto the column and the column was washed with 20 ml of binding buffer and then 20 ml of washing buffer. The protein was eluted with 10 ml of elution buffer.

Column 2: HP Q column (ion exchange), PI value of protein: 5.38

Buffers: Buffer A: 20 mM Tris-Cl pH 8.5, 50mM NaCl; Buffer B: 20 mM Tris-Cl pH 8.5, 2 M NaCl

Procedure: The ZADH1A protein was applied to 5ml HP Q column in buffer A and eluted from the column by a linear gradient with buffer B.

Enzymatic treatment: TEV cleaved.

Column 3: Ni-NTA (TEV clean up)

Buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP

Procedure: Total 5 mg of protein was cleaved with 300 µg of TEV protease at 4°C for 48 hours.

TEV clean up: The TEV cleaved protein was applied to a 1 ml Ni-NTA column, already equalibrated with gel filtration buffer (10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP). The flow through form the column was collected. The eluate from the column was monitored by SDS gel analysis.

Extraction

Procedure
Lysis buffer: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 5 mM Imidazole, Complete® protease inhibitors (Roche, 1 tbl/50 ml). Frozen cell pellets were thawed and resuspended in a total volume of 30-40 ml of lysis buffer, and disrupted by using Avestin C-5 microfluidizer, and a supernatant containing the target protein was obtained by centrifugation at 21,000 (rpm) for 45 minutes.
Concentration: The ZADH1A protein (in buffer; 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP) was concentrated to 5 mg/ml using Vivaspin 10K concentrators and stored at -80oC.
Ligand
MassSpec: Corresponds to theoretical mass, as determined by ESI-TOF MS.
Crystallization: Crystals were grown by vapour diffusion in sitting drops at 20°C. Before setting up the experiment NADP was added to the protein to a final concentration of 5mM. A sitting drop consisting of 75 nl protein and 100 nl well solution was equilibrated against well solution containing 2.4 M of Na-Malonate pH 7.0. The crystals were mounted directly from the drop using Na-Malonate as a cryoprotectant and flash-cooled in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Resolution: 2.4 Å, X-ray source: Synchrotron SLS-X10SA, single wavelength.
Data Processing: