UGDH

PDB:2QG4

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|4507813
Entry Clone Source:MGC
SGC Clone Accession:UGDHA-c103
Tag:N terminal SET1-tag, SBP-tag, TEV protease site (indicated by *)
mdpeeasvtsteetltpaqeaartraank arkeaelaaataeqtsdekttgwrgghvv eglageleqlrarlehhpqgqrepsggck lglgtenlyfq*sm
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

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mdpeeasvtsteetltpaqeaartraankarkeaelaaataeqtsdekttgwrgghvveglageleqlrarlehhpqgqrepsggck
lglgtenlyfq*smFEIKKICCIGAGYVGGPTCSVIAHMCPEIRVTVVDVNESRINAWNSPTLPIYEPGLKEVVESCRGKNLFFSTN
IDDAIKEADLVFISVNTPTKTYGMGKGRAADLKYIEACARRIVQNSNGYKIVTEKSTVPVRAAESIRRIFDANTKPNLNLQVLSNPE
FLAEGTAIKDLKNPDRVLIGGDETPEGQRAVQALCAVYEHWVPREKILTTNTWSSELSKLAANAFLAQRISSINSISALCEATGADV
EEVATAIGMDQRIGNKFLKASVGFGGSCFQKDVLNLVYLCEALNLPEVARYWQQVIDMNDYQRRRFASRIIDSLFNTVTDKKIAILG
FAFKKDTGDTRESSSIYISKYLMDEGAHLHIYDPKVPREQIVVDLSHPGVSEDDQVSRLVTISKDPYEACDGAHAVVICTEWDMFKE
LDYERIHKKMLKPAFIFDGRRVLDGLHNELQTIGFQIETIGKKV
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Vector:pBEN1-SGC.

Growth

Medium:TB
Antibiotics:
Procedure:10ml of an overnight culture was added into 1L of TB supplemented with 50 µg/ml of Ampicillin (total 8L). The cells were cultured at 37°C until the OD600 reached 1.8; the temperature was then decreased to 18°C. IPTG was added to 0.5mM final concentration, and the culture was continued at 18°C overnight.

Purification

Procedure
Column 1: Streptavidin agarose
Column 2: Superdex 200 Hiload 16 60

The Streptavidin agarose (10 ml) was thoroughly washed with 1L of binding buffer. The supernatant was incubated with the washed resin at 4°C for 1 hour by gentle rotation and packed into the column. The column was washed with 2x 50 ml of washing buffer. The protein was eluted with 10 ml of elution buffer and collected in 1.5 ml fractions.

An AKTA Purifier system (GE Healthcare) was used and run at 4°C. Fractions were analysed by SDS - PAGE and the most purified fractions were collected, concentrated and utilized for crystallization. .

Enzymatic treatment: For tag removal, 100 m l of TEV protease (6mg/ml) was added to the sample, incubated at 4°C overnight, before a final round of gel filtration chromatography. Purity was analyzed by SDS/PAGE and ESI mass spectrometry.

Extraction

Procedure
The cells were harvested by centrifugation at 4,000 g for 10 min. The pellet from 1 L culture was resuspended in 25 ml of extraction buffer. The sample was sonicated and then centrifuged at 37.500 g. The supernatant was kept for further purification.
Concentration:20 mg/ml.
Ligand
MassSpec: Experimental mass (ESI TOF mass spectrometry) 52018 Da; (52020 Da theoretical mass)
Crystallization: Crystals were grown by vapor diffusion in sitting drops at 4°C. Before setting up the experiment 5 mM NADH and 10 mM UDP-glucose (substrate complex) or 5 mM NAD+ and 1 mM UDP-glucuronate (product complex) were added to the protein. A sitting drop consisting of 150 nl protein (20 mg/ml) and 150 nl well solution was equilibrated against well solution containing 16% PEG 3350, 8% ethylene glycol, 160 mM NaBr, 80 mM BTP 6.5 (substrate complex) or 20% PEG3350, 10% ethylene glycol, 200 mM NaBr, 100 mM BTP, pH 6.5 (substrate complex). The crystals were transferred to a cryo-protectant consisting of well solution supplemented with 15% ethylene glycol and 3 mM UDP-glucose (substrate complex) or a solution of 70% (w/v) glucose mixed 1:4 with well solution (product complex), mounted in a loop, and flash-cooled in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Resolution: 2.1 Å, X-ray source: Synchrotron SLS-X10SA, single wavelength.
Data Processing: The structure was solved by molecular replacement using the structure 2o3j as a search model.