UEV3 LDH domain

PDB:2I6T

Revision

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Entry Clone Accession:
Entry Clone Source: Open Biosystems
SGC Clone Accession: ubc81.171.471; plate SDC092:H7
Tag:N-terminal His-tag with integrated TEV-cleavage site: MHHHHHHSSGRENLYFQG.
Host:E.coli BL21 (DE3)

Construct

Prelude:
Sequence:

MHHHHHHSSGRENLYFQGSKSWANHENKTVNKITVVGGGELGIACTLAISAKGIADRLVLLDLSEGTKGATMDLEIFNLPNVEISKD
LSASAHSKVVIFTVNSLGSSQSYLDVVQSNVDMFRALVPALGHYSQHSVLLVASQPVEIMTYVTWKLSTFPANRVIGIGCNLDSQRL
QYIITNVLKAQTSGKEVWVIGEQGEDKVLTWSGQEEVVSHTSQVLSNRAMELLRVKGQRSWSVGLSVADMVDSIVNNKKKVHSVSAL
AKGYYDINSEVFLSLPCILGTNGVSEVIKTTLKEDTVTEKLQSSASSIHSLQQQLKL

Vector:p28a-mhl

Growth

Medium:
Antibiotics:
Procedure:UEV3 LDH domain was expressed in E. coli BL21 (DE3) grown in Terrific Broth (Sigma) in the presence of 50 µg/ml of kanamycin at 37ºC to an OD600 of 5.5-6. Protein expression was then induced by isopropyl-1-thio-D-galactopyranoside at a final concentration of 0.05 mM, and incubation continued overnight at 15ºC. The culture was centrifuged; the cell pellets were collected, frozen with liquid nitrogen and stored at -80ºC.

Purification

Procedure
Column 1: TALON metal-affinity resin column (BD Biosciences) .
Column 2: HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham)

The cleared lysate was loaded onto a TALON metal-affinity resin (BD Biosciences) column at 4ºC. The column was washed with wash buffer A, wash buffer B and again wash buffer A, and the protein was eluted with elution buffer. The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with 20 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 2 mM dithiothreitol and concentrated by ultrafiltration.

Extraction

Procedure
The cell pellet was resuspended in lysis buffer and lysed using Microfluidizer (peak pressure 18,000 psi). The lysate was cleared by centrifugation.
Concentration:
Ligand
MassSpec:
Crystallization: Purified UEV LDH domain was crystallized using the hanging-drop vapor-diffusion method. Crystals grew when the protein (6 mg/ml in the above gel-filtration buffer) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 26% PEG3350, 0.2 M ammonium sulfate, 0.1 M sodium cacodylate, pH 5.2 at 293K temperature.
NMR Spectroscopy:
Data Collection:
Data Processing: