HDGF2

PDB:3QBY

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:48255931
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQG
Host:*E.coli* BL21 (DE3) codon plus RIL (Stratagene)

Construct

Prelude:
Sequence:

gMPHAFKPGDLVFAKMKGYPHWPARIDDIADGAVKPPPNKYPIFFFGTHETAFLGPKDLFPYDKCKDKYGKPNKRKGFNEGLWEIQN
NPHASYS

Vector:pET28-MHL

Growth

Medium: Terrific Broth
Antibiotics:50 µg/ml of kanamycin
Procedure:HDGF2 was expressed in *E.coli* BL21 (DE3) codon plus in Terrific Broth medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37 °C to an OD_{600} of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM and incubated overnight at 15 °C.

Purification

Procedure

The lysate was loaded onto 5 ml HiTrap column (Amersham Biosciences), charged with Ni^{2+}. The column was washed with 10 CV of wash buffer and the protein was eluted with elution buffer. The protein was dialyzed against dialysis buffer. TEV protease was added to combined fractions containing HDGF2. The cut and uncut protein of HDGF2 were separated on a Ni column. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM PIPES, pH 6.5, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 8 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80 degrees Celsius. For the purification the cell paste was thawed and resuspended in lysis buffer with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:34.4 mg/ml
Ligand
MassSpec: Expected MW is 10696.15 Da
Measured MW is 10696.4774 Da
Crystallization: Purified HDGF2 was complexed with H4K20me3 peptide (AKRHRKme3VLRDN) and crystallized using hanging drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution (11.6 mg/mL) with 1 µl of the reservoir solution containing 2.5 M ammonium sulfate, 0.1 M sodium acetate, pH 4.6.
NMR Spectroscopy:
Data Collection:
Data Processing: