UHRF1

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Revision

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SGC Clone Accession:ubh12.126.285.130H02 (SDC130H02)
Tag:N-terminal: MHHHHHHSSGRENLYFQG
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgMWDETELGLYKVNEYVDARDTNMGAWFEAQVVRVTRKAPSRDEPCSSTSRPALEEDVIYHVKYDDYPEN
GVVQMNSRDVRARARTIIKWQDLEVGQVVMLNYNPDNPKERGFWYDAEISRKRETRTARELYANVVLGDDSLNDCRIIFVDEVFKIE
RPGE

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure: The protein was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 Âµg/ml of kanamycin at 37ÂºC to an OD600 of 7.5. Protein expression was induced 0.05 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15ÂºC. The culture was centrifuged (12,000 x g, 15 minutes) and cell pellets collected and stored at -80ÂºC. In order to obtain the selenomethionyl derivative of the UHRF1 SRA domain, the cells were grown in M9 medium supplemented with glycerol using a M9 SeMET High-Yield growth media kit package (MD045004-50L, Medicilon) according to manufacturerÂ□s instructuion and the protein was purified as below.

Purification

Buffers
Wash buffer A: 10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 10 mM imidazole, 1 mM β-mercaptoethanol
Wash buffer B: Wash buffer A plus 0.05% Tween 20
Elution buffer: 10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 200 mM imidazole, 1 mM

β-mercaptoethanol
Gel Filtration buffer: 20 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 2 mM dithiothreitol
Ion-exchange buffer A: 20 mM Tris-HCl, pH 8.0, 5% glycerol, 2 mM DTT
Ion-exchange buffer B: 20 mM Tris-HCl, pH 8.0, 1 M NaCl, 5% glycerol, 2 mM DTT
Procedure
The cleared lysate was loaded onto a 3 mL TALON metal-affinity resin column (BD Biosciences) at 4ºC. The column was washed with 10 mL Wash buffer A, 10 mL Wash buffer B, and 10 mL Wash buffer A. The protein was eluted with 6 mL Elution buffer. The N-terminal His-tag was removed by overnight incubation of the protein with TEV protease at 4ºC.

The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with Gel Filtration buffer. Fractions containing protein (analyzed by SDS-PAGE) were pooled.

Final purification was achieved by ion-exchange chromatography on a 5-ml HiTrapQ column using linear 0 Â 50 % gradient of Ion-exchange buffer B in Ion-exchange buffer A. The target protein eluted around 30% buffer B. The corresponding fractions were combined and protein was concentrated by ultrafiltration using an Amicon Ultra centrifugal filter with 10 kD cutoff to a final concentration of 28 mg/ml.

The yields of the protein and its selenomethionyl derivatives were approx. 7 and 9 mg per liter of bacterial culture, respectively.

Extraction

Buffers
Lysis buffer: 10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 2 mM imidazole, 1 mM b-mercaptoethanol, 0.1 ÂµM phenylmethyl sulfonyl fluoride (PMSF)
Procedure
The cell pellet from a 2 L culture was resuspended in 50 ml Lysis buffer, lysed using a Microfluidizer at 18,000 PSI, and cleared by centrifugation at 40,000 x g for 30 min.
Concentration:
Ligand
MassSpec: Mass-spectroscopy by LC/MS showed pure product of correct molecular weight corresponding selenomethionyl derivative of the tandem-Tudor domain with addition of an N-terminal glycine residue from the tag and all four methionine residues substituted with selenomethionine.
Crystallization: Purified protein (10 mg/ml) was mixed in 1: 100 molar ratio with a peptide, TARKme3ST, corresponding to residues 6 Â 11 of histone H3 with Lys9 trimethylated. Crystals of the UHRF1 tandem-Tudor domain in complex with the peptide were grown at 298 K using the hanging drop method by mixing 1 volume of the protein-peptide mixture with 1 volume of well solution consisting of 3 M sodium formate and 0.1 M sodium acetate, pH 5.0. The crystals were cryoprotected by immersion in the well solution mixed in 1:1 ratio with a water solution containing 20% (w/v) sucrose, 4% (w/v) glucose, 18% (v/v) glycerol and 18% (v/v) ethylene glycol.
NMR Spectroscopy:
Data Collection:
Data Processing: