Human NRDP1 + USP8 complex

PDB:2GWF

Revision

Revision Type:created
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Entry Clone Accession:
Entry Clone Source:
SGC Clone Accession:NRDP1: nrdp1.193.317; plate SDC046:F7; USP8: usp08.0181.0319, plate SDC051:H2
Tag:N-terminal his tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPR*GS
Host:

Construct

Prelude:
Sequence:

USP8:

mgsshhhhhhssglvprgsGAITAKELYTMMTDKNISLIIMDARRMQDYQDSCILHSLSVPEEAIS
PGVTASWIEAHLPDDSKDTWKKRGNVEYVVLLDWFSSAKDLQIGTTLRSLKDALFKWE
SKTVLRNEPLVLEGGYENWLLCYPQYTTNAKVT
NRDP1:
MGSSGLVPRGSTIEYNEILEWVNSLQPARVTRWGGMISTPDAVLQAVIKRSLVESGCPASI
VNELIENAHERSWPQGLATLETRQMNRRYYENYVAKRIPGKQAVVVMACENQHMGDD
MVQEPGLVMIFAHGVEEI
Vector:p28a-LIC

Growth

Medium:TB
Antibiotics:
Procedure: The proteins were expressed independently in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 microG/ml of kanamycin at 37degC to an OD600 of 7.5. Cells

were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15degC. The culture was centrifuged and the cell pellets were collected and stored at -80degC.

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 column from BD Biosciences at 4degC. The column was washed with wash buffer A, wash buffer B and again wash buffer A, and the protein was eluted with 10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 200 mM imidazole, 1 mM ß-mercaptoethanol. 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, 2mM dithiothreitol and concentrated by ultrafiltration.

Extraction

Procedure
The cell pellets were resuspended in lysis buffer and inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF) and lysed using Microfluidizer. The lysate was cleared by centrifugation.
Concentration:
Ligand
MassSpec:
Crystallization: The proteins were mixed in 1:1 ratio and crystallized by means of hanging drop vapor diffusion under the following conditions:12% PEG5000 MME, 0.1 M bis-tris, 1 MM DTT, pH 6.5, 298 K.
NMR Spectroscopy:
Data Collection:
Data Processing: