USP8

PDB:3N3K

Revision

Revision Type:created
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Entry Clone Accession:usp08.NM_005154.ORI.AB1489_E06.vec
Entry Clone Source: OriGene
SGC Clone Accession:
Tag:N-terminal Tag: MGSSHHHHHHSSGLVPRGS
Host: Bacterial cells

Construct

Prelude:
Sequence:

PTVTPTVNRENKPTCYPKAEISRLSASQIRNLNPVFGGSGPALTGLRNLGNTCYMNSILQCLCNAPHLADYFNRNCYQDDINRSNLL
GHKGEVAEEFGIIMKALWTGQYRYISPKDFKITIGKINDQFAGYSQQDSQELLLFLMDGLHEDLNKADNRKRYKEENNDHLDDFKAA
EHAWQKHKQLNESIIVALFQGQFKSTVQCLTCHKKSRTFEAFMYLSLPLASTSKCTLQDCLRLFSKEEKLTDNNRFYCSHCRARRDS
LKKIEIWKLPPVLLVHLKRFSYDGRWKQKLQTSVDFPLENLDLSQYVIGPKNNLKKYNLFSVSNHYGGLDGGHYTAYCKNAARQRWF
KFDDHEVSDISVSSVKSSAAYILFYTSLG

Vector:pET28a-LIC

Growth

Medium:TB (Sigma T0918) supplemented with 150 mM glycerol, 100 µM Kanamycin and 600 µl antifoam 204 (Sigma A-8311).
Antibiotics:
Procedure: Competent BL21 (DE3) cells (Invitrogen C6000-03) were transformed and grown using the LEX system (Harbinger BEC) at 37°C in 2L bottles (VWR 89000-242) containing 1800 ml of TB (Sigma T0918) supplemented with 150 mM glycerol, 100 µM Kanamycin and 600 µl antifoam 204 (Sigma A-8311). When OD(600) ~ 6 was reached, the temperature was reduced to 15°C, and one hour later protein expression was induced with 100 µM IPTG (BioShop IPT001)

and the culture was incubated overnight (16 hours) at 15°C. Cell pellets were collected by centrifugation (12,227 x g, 20 min), frozen and stored at -80°C.

Purification

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 protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare) using Gel Filtration Buffer. Fractions containing protein corresponding to the USP8 peak were pooled and concentrated by ultrafiltration to the final protein concentration of 7.6 mg/ml. The yield of the protein was 3 mg per litre bacterial culture.

Extraction

Procedure
After resuspension in 30 mL per litre bacterial culture of Lysis Buffer, cells were lysed using a Microfluidics M110-EH microfluidizer at 18,000 psi.
Concentration:
Ligand
MassSpec:
Crystallization:USP8 solution (50 µl, 7.6 mg/ml) was mixed with Ubv.8.2 (30 µl, 3.5 mg/ml), which resulted in USP8 : inhibitor molar ratio of 1:1. Before setting crystallization plate, the mixture was incubated for 1 h at ambient temperature (294 K) followed by incubation for 16 h at 281 K. Crystals of the USP8-inhibitor complex were grown at 291 K using the hanging drop method by mixing equal volumes of the above complex solution and Crystallization Buffer (24% polyethyleneglycol 3350, 0.1 M bis-Tris, pH 6.0, 0.2 M ammonium acetate and 0.5 mM dithiothreitol). The crystals were cryoprotected by immersion in the Crystallization Buffer mixed (1:1, v/v) with cryoprotecting mixture that consisted of 20% (w/v) sucrose, 4% (w/v) glucose, 18% (v/v) glycerol and 18% (v/v) ethylene glycol in water and placed in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data from a crystal of the USP8 catalytic domain in complex with Ubv.8.2 inhibitor was collected on a MAR-300 detector at the Canadian Light Source beamline CMCF 08ID-1. The data set was integrated and scaled using the HKL2000 program suite. The structure was solved by molecular replacement techniques using the program PHASER and search model PDB entry 2GFO and 3MTN. Iterative model building using the graphics program Coot and refinement package REFMAC5 led to a model with an R factor of 17.8 (R_{free} 24.2%) for data between 35-2.6 Å.
Data Processing: