USP21

PDB:3I3T
Entry Clone Source: Open Biosystems
SGC Clone Accession:usp21.209.563.122G08 (SDC122G08)
Tag:N-terminal tag: MGSSHHHHHHSSGLVPRGS
Host:BL21 (DE3)
Vector:pET28a-LIC

Sequence:

mgsshhhhhhssglvprgsSGHVGLRNLGNTCFLNAVLQCLSSTRPLRDFCLRRDFRQEVPGGGRAQELTEAFADVIGALWHPDSCE
AVNPTRFRAVFQKYVPSFSGYSQQDAQEFLKLLMERLHLEINRRGRRAPPILANGPVPSPPRRGGALLEEPELSDDDRANLMWKRYL
EREDSKIVDLFVGQLKSCLKCQACGYRSTTFEVFCDLSLPIPKKGFAGGKVSLRDCFNLFTKEEELESENAPVCDRCRQKTRSTKKL
TVQRFPRILVLHLNRFSASRGSIKKSSVGVDFPLQRLSLGDFASDKAGSPVYQLYALCNHSGSVHYGHYTALCRCQTGWHVYNDSRV
SPVSENQVASSEGYVLFYQLMQEPPR

Growth

Medium:TB

Procedure: Competent BL21 (DE3) cells (Invitrogen C6000-03) were transformed and grown using the LEX system (Harbinger BEC) at 37 degC 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 OD600 = ~6 was reached, the temperature was reduced to 15 degC, and one hour later protein expression was induced with 100 µM IPTG (BioShop IPT001) and the culture was incubated overnight (16 hours) at 15 degC. Cell pellets were collected by centrifugation (12227 xg, 20 min), frozen and stored at -80 degC.

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 N-terminal His-tag was removed by overnight incubation of the protein with thrombin (1 unit/mg protein) at 4°C. 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 Usp21 peak were pooled and concentrated by ultrafiltration. The yield of the protein was 6 mg per liter of bacterial culture.

Modification with Suicide Substrate: Purified catalytic domain of Usp21 (21 mg) was incubated with 10-fold molar excess of ubiquitin(1-75)-bromoethylamide for 45 min at room temperature (21°C). The covalent complex was purified by gel-filtration chromatography as above, concentrated by ultrafiltration to a volume of 0.5 ml and diluted with 9.5 ml Hydrophobic

Interaction Buffer A. The sample was applied on 5-ml HiTrap Phenyl HP column (GE Healthcare), and the column was washed with 25 ml Hydrophobic Interaction Buffer A. The covalent Usp21-ubiquitin complex was eluted using a linear gradient (0 - 100%) of Hydrophobic Interaction Buffer B. One-ml fractions were collected, and those corresponding to the major chromatographic peak were combined and concentrated by ultrafiltration to a final protein concentration of 10 mg/ml. The concentrated protein was stored on ice. Coomassie-stained SDS-PAGE showed that the product was pure and analysis by LC/MS showed that its molecular weight was close (within 4.6 Da) to the calculated molecular weight of the covalent ubiquitin 1-75 complex of the Usp21 catalytic domain without N-terminal His-tag and with ethylene spacer between the two proteins

Extraction

Procedure: After resuspension in 30 mL per liter bacterial culture of Lysis Buffer, cells were lysed using a Microfluidics M110-EH microfluidizer at 18,000 psi.

Structure Determination

Crystallization: Crystals of the covalent ubiquitin complex of Usp21 catalytic domain were grown at 298 K using the hanging drop method by mixing equal volumes of protein solution (10 mg/ml) and Crystallization Buffer (13% polyethyleneglycol 3350, 0.1 M bis-Tris, pH 6.5, 0.1 M ammonium sulfate, 5 mM TCEP and 20% (v/v) 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). The crystals were cryoprotected by immersion in the Crystallization Buffer supplemented with additional 20% (v/v) of the above cryoprotecting mixture and placed in liquid nitrogen.

Data Collection: Diffraction data from a crystal of the Usp21 catalytic domain was collected at beamline 23-ID-B of the GM/CA-CAT at the Advanced Photon Source (Argonne National Laboratory).

Data Processing: The data set was integrated and scaled using the XDS program package. The structure was solved by molecular replacement techniques using the program PHASER and search model PDB entry 2IBI. Iterative model building using the graphics program Coot and refinement package REFMAC5 led to a model with an R factor of 18.8 % (Rfree 21.8 %) for data between 50-2.59 Å. The coordinates and structure factors have been deposited on 2009.06.30 into the RCSB PDB database with ID code 3I3T