

USP8 catalytic domain

PDB:2GFO

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:

Entry Clone Source:

SGC Clone Accession:usp08.0734.1110; plate SDC0064:H8

Tag:

Host:

Construct

Prelude:

Sequence:

MGSSHHHHHSSGLVPRGSPTVTPTVNRNKPTCYPKAEISRLSASQIRNLNPVFGGSGPALTGLRNLGNTCYMNSILQCLCNAPH
ADYFNRNCYQDDINRSNLLGHKGEVAEEFGIIMKALWTGQYRYISP KDFKITIGKINDQFAGYSQQDSQELLFMDGLHEDLNKAD
NRKRYKEENNDHDDFKAAEHAWQKHQLNESIIVALFQGQFKSTVQCLTCHKKSRTEAFMYLPLASTSKCTLQDCLRLFSKEE
KLTDNNRFYCSHCRARRDSLKKIEIWKLPPVLLVHLKRF SYDGRWKQKLQTSVDFPLENLDLSQYVIGPKNNLKYNLFSVSNHYGG
LDGGHYTAYCKNAARQRWFKFDDHEVSDISVSSVKSSAAYILFYTSLG

Vector:pET-28a-LIC

Growth

Medium:

Antibiotics:

Procedure:The protein was expressed 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

Immobilized-metal Affinity Chromatography: An aliquot (20 microL) of clarified supernatant is reserved for later analysis by SDS-PAGE. To the rest of it, 6 mL of 50% suspension of TALON metal-affinity resin (BD Bioscience) in the lysis buffer is added (3 mL suspension per each 50-mL tube with the supernatant). The tubes are incubated for 1 h in refrigerator with constant stirring, centrifuged at 4°C for 2 min at 1,000 rpm in SX4750 rotor using Allegra X-12R centrifuge (Beckman Coulter) and the supernatant is discarded. The affinity resin pellets are suspended in 10 mL lysis buffer and transferred into an Econo-Column (Bio-Rad 732-1010). The buffer is allowed to drain and discarded. The settled gel is washed with 15 mL lysis buffer containing 10 mM imidazol (washing buffer) followed by 15 mL washing buffer containing 0.05% Tween 20 (Sigma P7949) and 30 mL washing buffer; the washings are discarded. Elution is achieved using lysis buffer containing 200 mM imidazol. First 0.5 mL eluate is discarded and two 5-mL fractions are collected after that in tubes containing 50 μ L 1M DTT (final DTT concentration is 10 mM). Protein concentration in these fractions is determined using Coomassie Plus Protein Assay reagent (Pierce 1856210) and bovine serum albumin (Pierce 23209) as a protein standard for calibration. The recombinant protein is usually eluted in the first 5-mL fraction and it is used directly at the next purification step. Some His-tagged proteins spread between the two fractions. In this case, the fractions are combined and concentrated down to approx. 5-mL volume by ultrafiltration (Amicon Ultra-15 10,000 MWCO, UFC901024 or 5,000 MWCO, UFC900524, as appropriate, Millipore).

Size-exclusion Chromatography: This step is performed using an AKTA Purifier or an AKTA Express system (GE Healthcare). The protein sample is loaded onto an XK16x65 column packed with HighLoad Superdex 200 (GE Healthcare) and equilibrated with 20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 5% glycerol, 10 mM dithiothreitol (BioShop Canada, DTT 001). Elution is performed with the same buffer at a flow-rate of 3 mL/min in the Peak Fractionation mode: {Slope: min. peak width 0.833 min; level 0.000 mAU; peak start slope 10.000 AU/min; peak end slope 20.000 AU/min}. Note: If target protein has OD₂₈₀ (0.1%, 1 cm) lower than 0.2, monitor the eluate absorbance at 215 nm and use either time/volume-based or manual fraction collection. Fractions corresponding to major peak on the chromatogram are combined and analyzed by SDS-PAGE and LC/MS. Protein concentration is determined by measuring UV absorbance of the combined fractions at 280 nm. Purified protein is concentrated using an ultrafiltration concentrator as above to a final concentration of approx. 50 mg/mL for further crystallographic screening (preceded by a PC test, Hampton Research, in order to determine optimal protein concentration) and biophysical characterization. The concentrated protein solution is divided into 100- μ L aliquots that are frozen by immersion in liquid nitrogen and stored at -80°C.

Extraction

Procedure

Concentration:

Ligand

MassSpec:

Crystallization: Crystals were obtained by hanging drop vapour diffusion in 18% PEG3350; 0.1 M bis-tris, pH 6.3, 0.2 M potassium succinate, 1 mM DTT, at 298 K.

NMR Spectroscopy:

Data Collection:

Data Processing: