MPDZ (11th domain)

PDB:2QG1

Revision

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Entry Clone Accession:gi|4505231
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Tag:N-terminal, TEV cleavable hexahistidine tag
Host:BL21(DE3)-R3-pRARE2 (A homemade phage resistant version of BL21(DE3) containing the pRARE2 plasmid from Rosetta II (DE3) cells).

Construct

Prelude:
Sequence:

smSDTLTIELQKKPGKGLGLSIVGKRNDTGVFVSDIVKGGIADADGRLMQGDQILMVNGEDVRNATQEAVAALLKCSLGTVTLEVGR
I*STYV*

Vector:pNIC28-Bsa4.

Growth

Medium:
Antibiotics:
Procedure:Transformation: The construct DNA was transformed into homemade chemically competent cells of the expression strain by a standard heat shock procedure.

Glycerol stock preparation: A number of colonies from the transformation were used to innoculate 1 ml of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day glycerol stocks were prepared from this overnight culture.

Expression: A glycerol stock was used to innoculate 50 ml of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day this starter culture was used to innoculate 2x 1L of TB media (17 ml starter culture into each) containing 50 µg/ml kanamycin. After 7 hours the temperature was reduced to 25°C (OD 6.2). After a further 1 hour the cells were induced by the addition of 0.5 mM IPTG. The expression was continued overnight.

Cell harvest: Cells were spun down at 4°C, resuspended in Lysis Buffer and placed in a -80°C freezer.

Purification

Procedure
Column 1: Ni-NTA (0.5 ml of resin).
Column 2: Gel filtration. Hiload S200 16/60 - 120 ml volume

The column was equilibrated with 10CV of Binding Buffer. The clarified cell extract was passed through the column twice. The column was then washed with 50 ml of Binding Buffer and 30 ml of Wash Buffer. The protein was eluted with 15 ml of Elution Buffer.

The gel filtration column was pre-equilibrated with Gel Filtration Buffer. The concentrated eluant from column 1 was loaded on the gel filtration column at a flow rate of 1.0 ml/min. Eluted proteins were collected in 1.8 ml fractions. The fractions containing protein were identified on a coomasie blue stained gel.

TEV protease digestion: The gel filtration fractions containing MPDZA were pooled and TEV protease was added. The digestion was left overnight at 4°C.
Rebinding of impurities to Ni-NTA: The protein was mixed with Ni-NTA resin (pre-equilibrated into Gel Filtration Buffer) at 4°C, the resin was spun down and the supernatent collected.

Extraction

Procedure
The cell pellet was resuspended in 40 ml of lysis buffer. PMSF was added. The resuspended cell pellet was lysed using an Emulsiflex C5 high-pressure homogeniser, collecting a final volume of approximately 50 ml after dilution with Lysis Buffer. PEI was added to a final concentration of 0.15 % and the cell debris and precipitated DNA were spun down.
Concentration: The TEV protease cleaved MPDZA was concentrated to 12 mg/ml (measured using a nanodrop machine), distributed into aliquots and frozen at -80°C.
Ligand
MassSpec: Measured: 9564 (-72, a glutamate to glycine mutation, E1727G, is observed in the crystal structure).
Expected: 9636.
Crystallization: Crystals grew from a 2:1 ratio of protein to precipitant solution (0.8 M ammonium sulphate, 0.1 M citrate pH 4.0), using the vapour diffusion method. Crystals were cryo-protected by equilibration into precipitant solution containing 20% ethylene glycol, and then flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Resolution: 1.5 Å, X-ray source: Synchrotron SLS-X10SA, single wavelength.
Data Processing: