FES

PDB:4DYL

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Mammalian Gene Collection (IMAGE consortium clone ID 5170548).
SGC Clone Accession:
Tag:N-terminal, TEV cleavable hexahistidine tag
Host:

Construct

Prelude:
Sequence:

MHHHHHHSSGVDLGTENLYFQSMGFSSELCSPQGHGVLQQMQEAELRLLEGMRKWMAQRVKSDREYAGLLHHMSLQDSGGQSRAISP
DSPISQSWAEITSQTEGLSRLLRQHAEDLNSGPLSKLSLLIRERQQLRKTYSEQWQQLQQELTKTHSQDIEKLKSQYRALARDSAQA
KRKYQEASKDKDRDKAKDKYVRSLWKLFAHHNRYVLGVRAAQLHHQHHHQLLLPGLLRSLQDLHEEMACILKEILQEYLEISSLVQD
EVVAIHREMAAAAARIQPEAEYQGFLRQYGSAPDVPPCVTFDESLLEEGEPLEPGELQLNELTVESVQHTLTSVTDELAVATEMVFR
RQEMVTQLQQELRNEEENTHPRERVQLLGKRQVLQEALQGLQVALCSQAKLQAQQELLQTKLEHLGPGEPPPVLLLQDD

Met1 to Asp405
The N-terminal residues, MHHHHHHSSGVDLGTENLYFQS, derive from the vector
Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:

Purification

Procedure
Cell Lysis: The resuspended cell pellet was thawed and lysed using a high pressure homogeniser. PEI (polyethyleneimine) was added to a final concentration of 0.15 %. The cell debris and precipitated DNA were spun down.Lysis Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5 mM Imidazole, 5% Glycerol, 0.5 mM TCEP, Protease inhibitor cocktail (Roche) and 15 U/mL benzonase. Purification: Column 1: 5 ml of Ni-Sepharose in a 2 cm diameter gravity flow column. Column 1 Buffers: Binding Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5 mM Imidazole, 5% Glycerol, 0.5 mM TCEP. Wash Buffer: As Binding Buffer except 25 mM

imidazole.Elution Buffer: As Binding Buffer except 250 mM imidazole. Column 1 Procedure: The clarified supernatant was passed through the column. The column was washed with 50 ml of Binding Buffer and Wash Buffer and 25 ml of Elute Buffer was passed through to elute the protein. Column 2: S75 16/60 Gel Filtration (GE Healthcare) Column 2 Buffers: GF Buffer: 20 mM Tris pH 7.4, 200 mM NaCl, 2 mM DTT Column 2 Procedure: The eluted protein was concentrated to 5 ml volume and injected onto the column.

Extraction

Procedure
Expression strain: BL-21(DE3)-R3-pRARE2 (A homemade phage resistant version of BL21(DE3)). Transformation: The construct DNA was transformed into homemade chemically competent cells of the expression strain by a standard heat shock procedure. Expression: Colonies were used to inoculate 2x 50 mL of TB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol in a 250 ml baffled shaker flask, which was placed in a 37°C shaker overnight. The next day 6x 10 mL of this starter culture was used to inoculate 6x 1L of TB media containing 50 µg/ml kanamycin in 2L baffled shaker flasks. When the OD600 was approximately 1.5, the temperature was reduced to 18°C and the cells were induced by the addition of 1 mM IPTG. The expression was continued overnight. Cell harvest: Cells were spun at 6000rpm for 10 mins and the pellets resuspended in Lysis Buffer and then frozen at -20°C
Concentration: The FES FBAR domain was concentrated to 19.8 mg/ml (measured by 280 nm absorbance).
Ligand
MassSpec: Expected: 46418Observed: 46419
Crystallization: The compound Phosphoinositol-3,4,5-triphosphate (PI(3,4.5)P3) was added to the concentrated protein sample to a concentration of 1 mM, although the compound was not visible in the resulting structure. Crystals grew from a 2:1 ratio of protein and precipitant solution (0.2M Sodium malonate, 0.1M BisTrisPropane pH 6.5, 20% PEG 3350, 10% Ethylene glycol), using the vapour diffusion method.

Selenomethione derivative preparation
Crystals of selenomethionine derivatised protein were used to solve the structure. This protein was expressed using a similar protocol as for the native protein except with M9 minimal media and selenomethionine added at the time of induction.The selenomethionine derivatised protein was purified similarly except with a gel filtration buffer of 20 mM Tris pH 7.4, 150 mM NaCl, 5% glycerol and 2 mM DTT.The observed MW indicated that 9x Se were incorporated into the protein.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected by equilibration into precipitant solution containing 20% ethylene glycol, and then flash frozen in liquid nitrogen. Data was collected at Diamond, beamline I24.
Data Processing: