TXNRD1

PDB:2CFY

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC018122
Entry Clone Source:MGC
SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*), N-terminal his6 tag.
Host:BL-21(DE3)R3 phage resistant

Construct

Prelude: Mutagenesis: The TXNRD1A gene was mutated to replace the selenocysteine UGA codon with a GGA, encoding a glycine residue (bold G in the protein sequence below). Site-directed mutagenesis was performed as described in Sarkar G and Sommer SS (1990) Biotechniques 8(4):404-7.
Sequence:

mhhhhhhssgvdlgtenlyfqsmNGPEDL PKSYDYDLIIIGGGSGGLAAAKEAAQYGK KVMVLDFVTPTPLGTRWGLGGTCVNVG CI PKKLMHQAALLGQALQDSRNYGWKVEETV KHDWDRMIEAVQNHIGSLNWGYRVALREK KVVYENAYGQFIGPHRIKATNNKG KEKIY SAERFLIATGERPRYLGIPGDKEYCISSD DLFSLPYCPGKTLVVGASYVALECAGFLA GIGLDVTVMVRSILLRGFDQD MANKIGEH MEEHGIKFIRQFVPIKVEQIEAGTPGRLR VVAQSTNSEEIIEGEYNTVMLAIGRDACT RKIGLETVGVKINEKTGK IPVTDEEQTNV PYIYAIGDILEDKVELTPVAIQAGRLLAQ RLYAGSTVKCDYENVPTTVFTPLEYGACG LSEEKAVEKFGEENI EVYHSYFWPLEWTI PSRDNNKCYAKIICNTKDNERVVGFHVLG PNAGEVTQGFAAALKCGLTKKQLDSTIGI HPVCAEVFTTLS VTKRSGASILQAGCGG

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Medium: TB + 50 µg/ml Kanamycin + 34 µg/ml chloramp . 2 x 1 liter TB in 2.5-L baffled flasks were inoculated with 10 ml overnight culture and grown at 37°C. The protein expression was induced with 1 mM IPTG at OD600 = 4 at 18°C over night. The cells were collected by centrifugation and frozen at -80°C.

Purification

Procedure
Column 1 : Ni-affinity, HisTrap, 1 ml (GE/Amersham)
The cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system

(GE/Amersham). The column was then washed with 10 volumes of lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280 was automatically collected.

Column 2 : Gel filtration: Hiload 16/60 Superdex 200 prep grade 120 ml, Code no. 17-1069-01 Amersham Biosciences
The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column in GF buffer at 0.80 ml/min. Eluted proteins were collected in 2 ml fractions.

Extraction

Procedure
Frozen cell pellets were thawed at 37°C and resuspended in a total volume of 100 ml lysis buffer. The cells were disrupted by high pressure (20 kpsi) followed by sonication.

Nucleic acids and cell debris were removed by adding 0.15% PEI , followed by centrifugation for 30 minutes at 40 000xg. The supernatant was then further clarified by filtration (0.45 µm).
Concentration: The protein was concentrated in Amicon (5K) to 25 mg/ml and the protein concentration determined with bradford using BSA as a standard.
Ligand
MassSpec: The mass for TXNRD1Ap002 was 57216 Da , in agreement with the predicted mass of 57214 for the his-tagged protein.
Crystallization: Plate-shaped crystals with a maximum dimension of 500 m m were grown by vapor diffusion in sitting drops at 4°C. A 660 nl drop comprised of 15 mg/ml protein, 300 mM NaCl, 180 mM NDSB-221, 6 mM HEPES pH 7.5, 6 mM DTT, 6% PEG 3350, 3 % glycerol, 0.3 mM TCEP, 30 mM Tris pH 7.0 was equilibrated against a well solution containing 20% PEG 3350, 0.1 M Tris pH 7.0. The crystal was transferred to a cryoprotectant consisting of 80% well solution 20% glycerol before flash-cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Resolution: 2.7Å, X-ray source: Synchrotron ALS -8.2.1, single wavelength.
Data Processing: