GPX4

PDB:2GS3

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC021567
Entry Clone Source:MGC
SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s(m), TEV-cleavable (*), N-terminal his6 tag.
Host: Rosetta-R3

Construct

Prelude: Mutagenesis: The GPX4 gene was mutated to replace the selenocysteine UGA codon with a GGA, encoding a glycine residue. Site-directed mutagenesis was performed as described in Sarkar G and Sommer SS (1990) Biotechniques 8(4):404-7. A truncated version of the mutated gene was successfully expressed and crystallized.
Sequence:

mhhhhhhssgvdlgtenlyfqsmRCARSM HEFSAKDIDGHMVNLDKYRGFVCIVTNVA SQGGKTEVNYTQLVDLHARYAECGLRI
LA FPCNQFGKQEPGSNEEIKEFAAGYNVKFD MFSKICVNGDDAHPLWKWMKIQPKGKGIL GNAIKWNFTKFLIDKNGCVVKRYG
PMEEP LVIEKDLPHYF

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 grown at 37°C. The protein expression was induced with 1 mM IPTG at OD600 = 3 at 18°C overnight. The cells were collected by centrifugation and frozen at -80°C.

Purification

Procedure
Column 1 : Ni-affinity, HisTrap, 1 ml (GE/Amersham Biosciences)
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 (GE/ Amersham Biosciences)
The eluted fractions from the Ni-affinity Histrap column were loaded on the gel filtration column at 1.0 ml/min. Eluted proteins were collected in 2 ml fractions.

Concentration: The protein was concentrated in Amicon (5 K) to 27 mg/ml and the protein concentration determined spectrophotometrically using the predicted molar extinction coefficient 32430 (M-1 cm-1).

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 further clarified by filtration (0.45 µm).
Concentration:
Ligand
MassSpec: The mass determined for GPX4Ap001 was 21151.8 Da, in agreement with the predicted mass for the his-tagged protein.
Crystallization: Crystals were grown by vapor diffusion at 20°C. A sitting drop consisting of 150 nl protein (27 mg/ml) and 150 nl well solution was equilibrated against well solution containing 20% PEG 3350, 0.2 M ammonium chloride, pH 6.3. The crystal was transferred to well solution supplemented with 15% 1,2 propanediol before flash-cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: