GPX2 - Human glutathione peroxidase 2

PDB:2HE3

Revision

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Entry Clone Accession:BC067221
Entry Clone Source:MGC
SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s(m), TEV-cleavable (*), N-terminal his6 tag
Host:

Construct

Prelude: Mutagenesis:The GPX2 gene was mutated to replace the selenocysteine UGA codon with TGT, encoding a Cys residue. Site-directed mutagenesis was performed by overlap extension using the polymerase chain reaction as described by Ho S.N. et al, (1989) Gene, v.77, p.51-59.
Sequence:

mhhhhhhssgvdlgtenlyfqsmIAKSFY DLSAINLDGEKVDFNTFRGRAVLIENVAS LCGTTTRDFTQLNELQCRFPRRLVVLG FP CNQFGHQENCQNEEILNSLKYVRPGGGYQ PTFTLVQKCEVNGQNEHPVFAYLKDKLPY PYDDPFSLMTDPKLIIWSPVRRSD VAWNF EKFLIGPEGEPFRRYSRTFPTINIEPDIK RLLKV

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 2x 10 ml overnight culture and grown at 37°C. The protein expression was induced with 1 mM IPTG at OD600 = 4.3 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)
Buffers: Lysis buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP. Wash buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 50 mM imidazole, 0.5 mM TCEP. Elution buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 350 mM imidazole, 0.5 mM TCEP.

Procedure: 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 : Gelfiltration, Hiload 16/60 Superdex 200 prep grade, 120 ml (GE/ Amersham Biosciences)
Buffers : 20 mM PIPES, pH 6.5, 500 mM NaCl, 50 mM Arg/ Glu, 0.5 mM TCEP.
Procedure: 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.

Concentration : The protein was concentrated in Amicon (5 K) to 2.1 mg/ml and the protein concentration determined spectrophotometrically using the predicted molar extinction coefficient 28420 (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 disrupted by high pressure (20 kpsi) and nucleic acids and cell debris removed by adding 0.15% PEI , followed by centrifugation for 30 minutes at 40 000xg. The supernatant was further clarified by filtration (0.20 μm).
Concentration:
Ligand
MassSpec: The mass determined for GPX 2Ap009 was 24085 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 75 nl protein and 75 nl well solution was equilibrated against well solution containing 20% PEG 3350, 200 mM sodium formate, 100 mM Bis-Tris propane pH 7.5, 10% ethylene glycol. The crystal was transferred to a cryoprotectant composed of well solution supplemented with 15% glycerol before flash-cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Resolution: 2.1 Å; X-ray source: Synchrotron SLS -X10.
Data Processing: