GLRX2: Human Glutaredoxin 2

PDB:2HT9

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:IMAGE 387
Entry Clone Source: Invitrogen
SGC Clone Accession:
Tag:
Host:E.coli strain Rosetta R3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmESNTSSSLENLATAPVNQIQETISDNCVVIFSKTSCSYCTMAKKLFHDMNVNYKVVELDLLEYG
NQFQDALYKMTGERTVPRIFVNGTFIGGATDTHRLHKEGKLLPLVHQCYLKKSKRKEFQ

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Medium: TB + 50 µg/ml Kanamycin + 34 µmg/ml chloramp.
2 x 1 liter TB in 2.5-L baffled flasks were inoculated with 2 x 10 ml overnight culture and grown at 37°C. The protein expression was induced with 1 mM IPTG at OD600 = 3.4 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 Biosciences)

Buffers: All buffers were degassed and purged with argon prior to usage. Lysis buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 10 mM imidazole, 10 mM GSH; Wash buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 50 mM imidazole, 10 mM GSH; Elution buffer: 50 mM potassium phosphate buffer, pH 7.5, 500 mM NaCl, 350 mM imidazole, 10 mM GSH

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 A280nm was automatically collected.

Column 2 : Hiload 16/60 Superdex 200 prep grade 120 ml (GE/Amersham Biosciences)

Buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 10 mM GSH

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 dimeric protein was concentrated in Amicon (5 K) to 11.5 mg/ml. The protein concentration was determined spectrophotometrically using the predicted molar extinction coefficient 8940 (M-1cm-1) and the protein stored under nitrogen to prevent oxidation.

Extraction

Procedure
Frozen cell pellets from 2 liter were thawed at 37°C and resuspended in a total volume of 150 ml degassed and argon purged lysis buffer. The cells were 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 40 000 x g. The supernatant was further clarified by filtration (0.20 mm).
Concentration:
Ligand
MassSpec: The mass determined was 16675.9 Da, in agreement with the predicted mass of 16678 for the his-tagged protein containing one intra-molecular disulfide.
Crystallization: Crystallization: Brown, hexagonal plate-shaped crystals (~75 µm) were grown in a glove box under argon to prevent oxidation of the iron-sulfur cluster. A sitting drop consisting of 1 ul protein and 1 ul well solution was equilibrated against well solution containing 1.6 M $MgSO_4$, 100 µm MES pH 6.5. The crystal was transferred to a cryoprotectant of 1.7 M sodium malonate pH 7.0 before flash-cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Resolution: 1.90Å; X-ray source: Synchrotron SLS -X10.
Data Processing: