Dimers of Glutaredoxin 2 as Mitochondrial Redox Sensors in Selenite-induced Oxidative Stress

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Fig. S 1. Determination of protein-bound glutathione

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Electronic supplementary information

Figure S 1. Determination of protein-bound glutathione. HeLa cells were treated for 18 h with 15 µM Sel. To analyze the monomerization of Grx2, mitochondrial cell fractions derivatized with 10 mM AIS, were subjected to Nu-PAGE in non-reducing conditions and WB analysis. The membrane was probed with an anti-Grx2 and an anti-GSH Ab. (a) Markers; (b) Cnt; (c) 15 µM Sel. (IB: immunoblot).

Figure S 2. Determination of Grx2 monomerization in cells after oxidative stress induction. HeLa cells were treated with a: Cnt; b: 1 mM BSO 48 h; c: 1 µM AF 3 h, d: 1 mM BSO 48 h + 1 µM AF 3 h. To analyze the monomerization of Grx2, mitochondrial cell fractions, derivatized with 10 mM AIS, were subjected to SDS-PAGE in non-reducing conditions and WB analysis. The densitometric analysis of the bands performed with NineAlliance software is reported in the right panel.
Figure S 3. Specific activity of TrxR1 and TrxR2 and of cytosolic and mitochondrial Grx in Sel treated cells. HeLa cells (3 x 10^7) were incubated for 3 (A), 6 (B), 12 (C) and 18 (D) h with 15 µM Sel or in control conditions and then subjected to cell sub-fractionation in order to obtain the cytosol and mitochondria enriched cell fractions. The cytosolic fraction was checked for TrxR1 (blue bars) and total glutaredoxin (red bars) activities; the mitochondrial fraction was probed for TrxR2 (gray bars) and Grx2 (orange bars) activities. The graphs reports the specific activity of each enzyme, expressed as nmol/min/mg protein, in Sel treated or control cells. a: TrxR1 Cnt; b: TrxR1 15 µM Sel; c: TrxR2 Cnt; d: TrxR2 15 µM Sel; e: cytosolic Grx Cnt; f: cytosolic Grx 15 µM Sel; g: mitochondrial Grx Cnt; h: mitochondrial Grx 15 µM Sel. The graph shows the mean ± SD of 3 experiments (*p<0.05, ***p<0.001).
Figure S 4. Iron distribution in the two mitochondrial fractions obtained after treatment with \textit{meta}-phosphoric acid. HeLa cells (3 x 10^7) were treated with 15 μM Sel for 18 h and then processed to obtain mitochondria following the protocol of Clayton and Shadel (see Experimental section). Mitochondria were then treated with 600 μL of 6% \textit{meta}-phosphoric acid for 20 min at 4°C in order to extract the labile iron pool. At the end of incubation, samples were centrifuged at 15800g for 10 min at 4°C. Both the supernatants (Acid-labile iron pool) and the pellets (Protein-bound (Heme) iron pool) were subjected to mineralization and analysis of the Fe content. The percentage of iron measured in the two fractions (mean ± SD of 4 experiments) is reported.
Figure S 5. FACS analysis of lipid peroxidation in HeLa cells treated with Sel +/- BHT using C11-BODIPY dye. HeLa cells (4.5 x 10^5) were treated with 15 μM Sel for 18 h with or without pre-incubation with 0.1 mM butylated hydroxytoluene (BHT) for 2 h and then subjected to the analysis of lipid peroxidation using C11-BODIPY dye as described in Experimental. Treatment of cells with 2.5 mM cumene hydroperoxide + 5 μM hemin (Chp + Hem) for 15 min is displayed as a positive control of lipid peroxidation induction.
Figure S 6. Release of Cyt C after Sel treatment in HeLa cells

(A, A') Detection of Cyt c remaining in the mitochondrial fraction. Cells (4.5 x 10⁵) were treated with 15 µM Sel for 18 h and then processed as reported in the Experimental section. (A) Aliquots of 10 µg protein of the mitochondrial fractions were subjected to SDS-PAGE (15%) followed by Western blot using a Cyt c monoclonal antibody. TOM20 content was determined as a loading control. (A') Relative amount of Cyt c in the mitochondrial fraction of control (a) or Sel-treated (b) HeLa cells showing a decrease of Cyt c in Sel-treated mitochondria. The graph reports the mean ± SD of 3 experiments (*p<0.05).

(B) Estimation of the purity of the cytosolic cellular fraction (absence of mitochondrial residues) through the analysis of the amount of the mitochondrial marker TOM20. (B') Densitometric analysis of the Western blot bands reported in B utilizing the Nine-Alliance software. Control (a, a'); Sel-treated (b, b').
Figure S 7. Western blot analysis of GPx4 in mitochondria samples in the presence of 15 μM Sel. (A) Level of GPx4 decreases in mitochondria of HeLa cells treated with 15 μM Sel for 18 h. In the same, the detection of TOM20 was performed as loading control. (B) Densitometric analysis of the Western blot bands reported in A utilizing the ImageJ software. a: Cnt; b: 15 μM Sel.