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PROTEIN-PROTEIN INTERACTION BETWEEN GLYOXALASE II AND SPECIFIC REDOX DEPENDENT PROTEINS THROUGH S-GLUTATHIONYLATION MODIFICATION.

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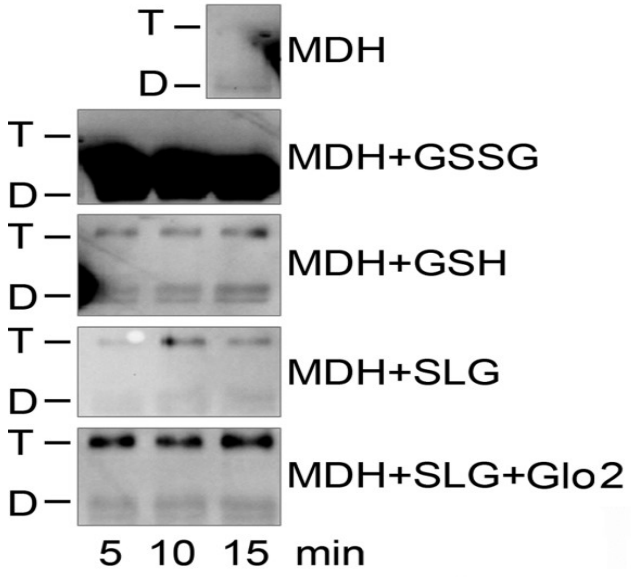
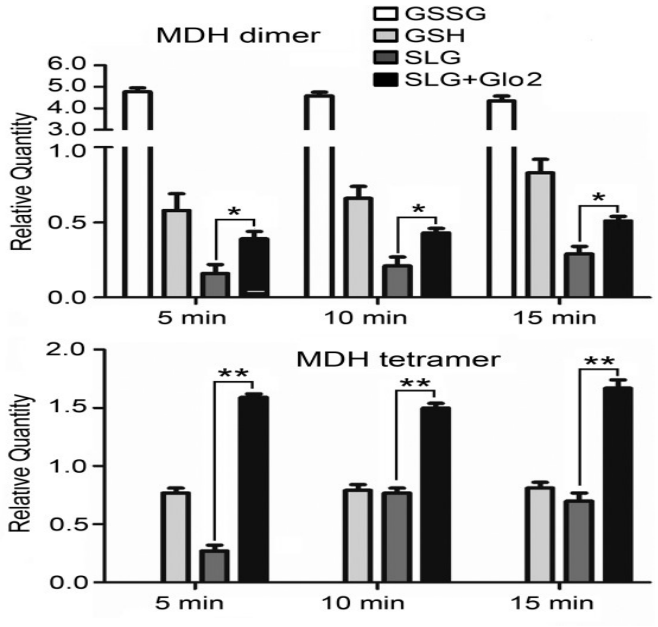
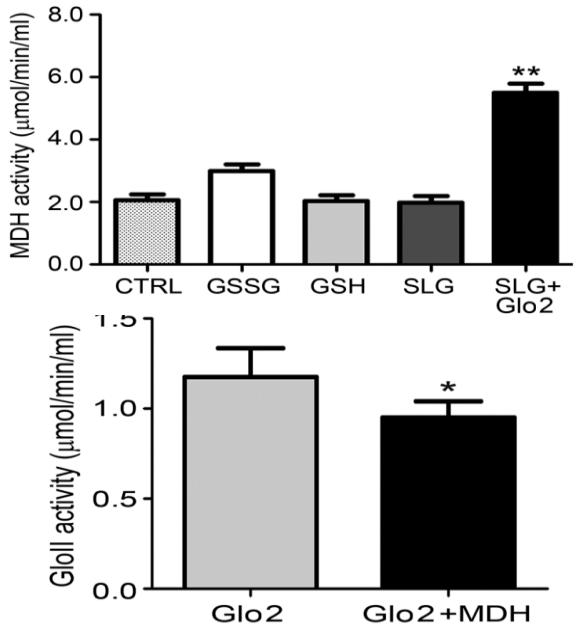
Background

S-glutathionylation involves the reversible formation of a mix disulphide-bridge between specific cysteine and a molecule of glutathione, the major non-protein antioxidant compound in the cell. Mechanism of glutathionylation can be spontaneous or catalyzed. Glyoxalase II (Glo2) enzyme was studied as a new potential candidate to promote S-glutathionylation. To demonstrate its active involvement in protein glutathionylation were used Actin, Cytochrome c (CYT-C); Malate Dehydrogenase (MDH) and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) purified proteins, which are known to be glutathionylated, for *in vitro* experiments. This work shows active involvement of cytosolic Glo2 for *in vitro* protein S-glutathionylation, suggesting a new mechanism of protein-SSG formation.

Glyoxalase 2 promotes S-glutathionylation and tetramerization of malate dehydrogenase…

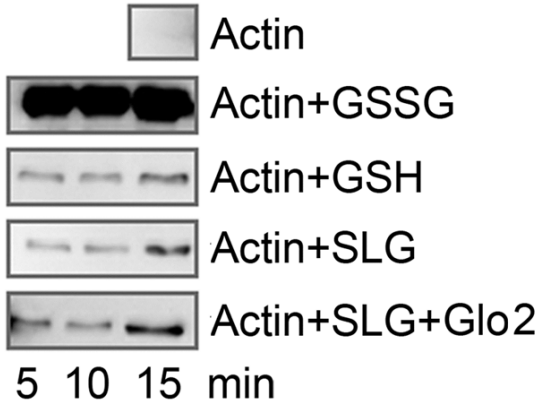


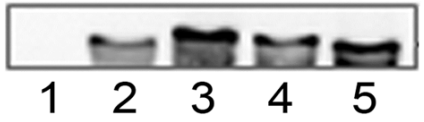




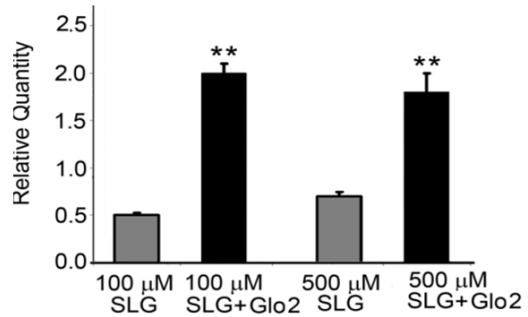
Mitochondrial enzyme MDH, showed a strong glutathionylation signal when incubated with Glo2 plus SLG, in particular Glo2 plus SLG is able to promote tetrameric conformation. MDH activity significantly increase in the presence of Glo2 plus SLG. Glo2 activity, instead, decrease in presence of MDH.

Glyoxalase 2 promotes S-glutathionylation of actin



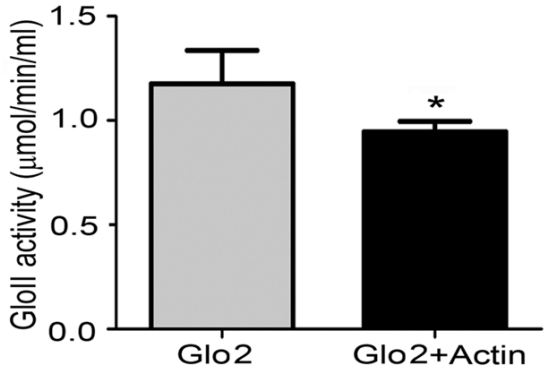


1. Actin
2. 100µM SLG 10 min
3. 100µM SLG plus GloII 10min
4. 500µM SLG 10 min
5. 500µM SLG plus GloII 10 min

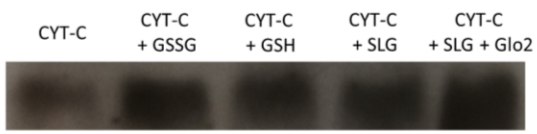


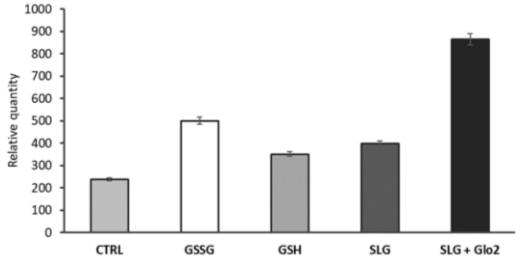
Actin incubated with SLG plus Glo2 show S –glutathionylation. When the concentration of SLG was lowered a greater difference in signal glutathionylation between spontaneous or catalyzed reaction was observed.

Glo2 activity decrease in presence of actin protein



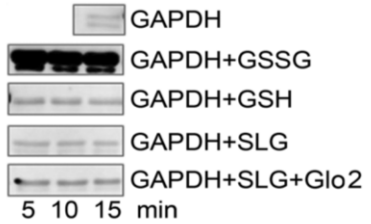
Glyoxalase 2 promotes S- glutathionylation of Cyt C



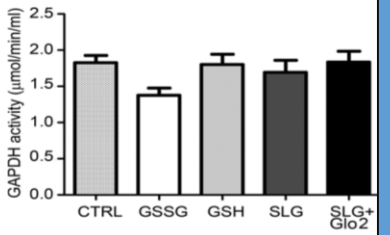


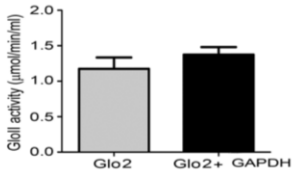
S-glutathionylation of Cyt c is higher in the presence of Glo2 plus SLG than Cyt c alone or plus GSH or GSSG.

Glyoxalase 2 not promotes S- glutathionylation of GAPDH



GAPDH showed weak glutathionylation band signal for the system Glo2plus SLG. Activity assay confirm the absence of glutathionylation of GAPDH by Glo2





Glo2 activity assay confirm the non implication in GAPDH glutathionylation

CONCLUSION:

This study shows that Glo2, through a specific interaction of its catalytic site with the target proteins, is able to perform a rapid and specific protein S-glutathionylation using its natural substrate SLG.

EXPERIMENTAL PROCEDURE:

Recombinant human cytosolic Glo2 was prepared by heterologous expression in Escherichia coli from a human adult liver cDNA library. Actin, MDH, Cyt C and GAPDH, were used to test in vitro S-glutathionylation by immunoblot with anti-GSH antibody. P value were calculated by Student's test.
Data were reported as mean SD, where n=3. P* value of < 0,05 was considered significant
P < 0,01and < 0,001 was highly significant.**

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