Polymorphisms of GST pi: Redox regulation through protein S-glutathionylation

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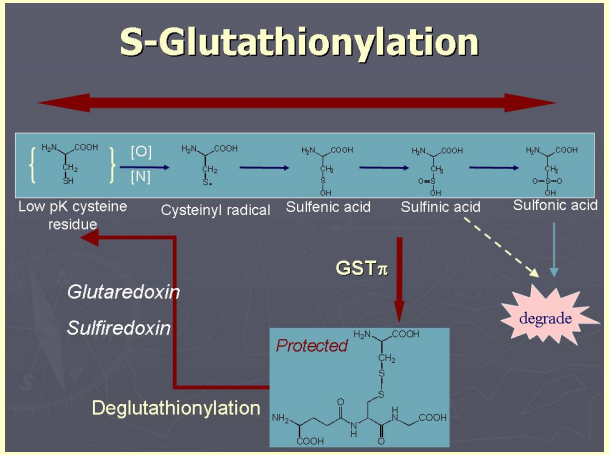
Abstract

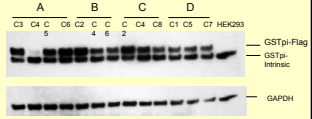
Glutathione S-transferase P1-1 (GSTP) is a prominent and ubiquitously expressed enzyme protein in many human cancers. Its expression is increased in response to a variety of apparently unrelated stress conditions and high levels (sometimes ~1-2% of cytosolic protein) are frequently linked to drug resistance phenotypes, even when the selecting drug is not a substrate for the enzyme. While recent results have shown that GSTP interacts non-covalently with kinases, it has always seemed probable that a more prevalent cellular function may be ascribed to such a common protein. Recently, we have demonstrated by *in vitro* and *in vivo* approaches that the catalytic properties of GSTP are critical to the post-translational S-glutathionylation of cysteine residues in a number of different target proteins (Townsend et al, JBC 2009). Genetic polymorphisms of GSTP have differing catalytic constants for some small molecule substrates. In the present studies, we evaluated the role of GSTP polymorphisms in regulating S-glutathionylation reactions. We generated stably transfected HEK293 cells expressing the four human GSTP*A-D alleles (Ile105/Ala114; Val105/Ala114; Val105/Val114; and Ile105/Val114) and a catalytically inactive mutant. In response to two drugs, NOV-002, a glutathione disulfide mimetic and PABA/NO, a nitric oxide releasing prodrug, cells mount a rapid S-glutathionylation of specific target proteins. The rate of protein S-glutathionylation is significantly enhanced by the presence of a catalytically active (intact tyr7 residue) GSTP and is also is greater for the GSTP*A and *D alleles. A role for GSTP in the catalytic formation of a disulfide between the thiolate of GSH and a low pK cysteine thiol in a target protein is a novel property for GSTP. The differential susceptibility to cancer initiation and drug response associated with some GSTP polymorphisms may be attributable to the their capacity to respond to oxidative and nitrosative stress through protein S-glutathionylation.

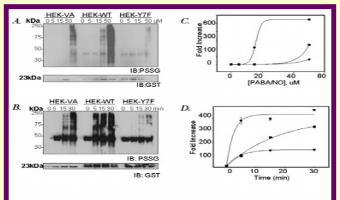


S-glutathionylation requires catalytically active GSTpi

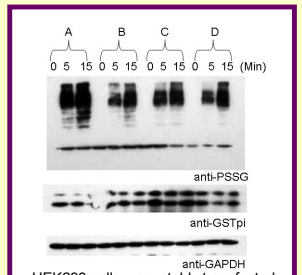
Catalytic activity of GSTP A-D

*GSTP1*A:* Ile105 ∞ Ala114 wild-type
*GSTP1*B:* Val105 ∞ Ala114 decrease
*GSTP1*C:* Val105 ∞ Val114 decrease
*GSTP1*D:* Ile105 ∞ Val114 no change





GSTπ enzymatic activity is crucial for protein S-glutathionylation. HEK-293 cells were transiently transfected with vector (HEK-VA), wild-type GSTπ (HEK-WT) or an enzymatically inactive mutant form of GSTπ (HEK-Y7F). Concentration- (A) and time-dependence (B) effects following PABA/NO treatment illustrate that increased ectopic expression of GSTπ stimulates, whereas mutation of the catalytic tyrosine in the enzyme active site diminishes S-glutathionylation (PSSG).



HEK293 cells were stably transfected with GSTPA-D vectors. Cells were treated with 5 uM PABA/NO for indicated times. Proteins were resolved by SDS-PAGE . P-SSG and GSTP expression were evaluated by immunoblot.

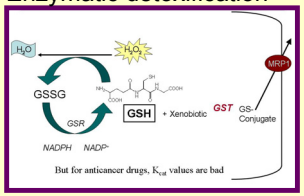
Background:

Functional Roles of GST

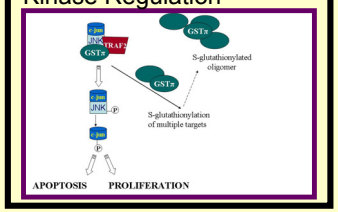
20th century
- Ligand binding/ transport (e.g. steroids; bilirubin; heme; NO)
- Enzymatic catalysis/ detoxification

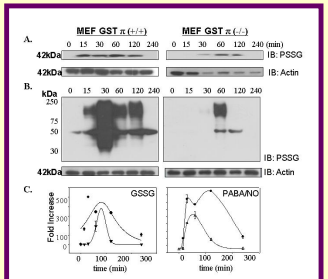
21st century
- Protein:protein interactions/ chaperones?
- Endogenous regulation of kinase mediated signaling
- Drug target linked to proliferative pathways
- Forward reaction in post-translational S-glutathionylation

Enzymatic detoxification



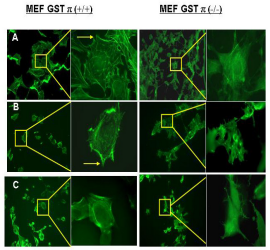
Kinase Regulation



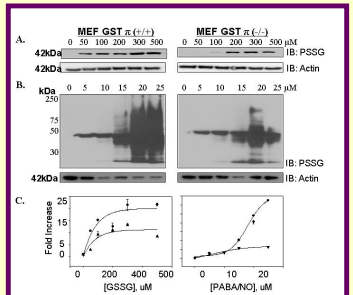


GSTπ expression enhances time-dependent S-glutathionylation of proteins. Mouse embryo fibroblast cells derived from GSTπ wild-type or knockout animals were treated with 200 μM GSSG (A) or 25 μM PABA/NO for 0 to 240 min (B). S-glutathionylation evaluated by immunoblot with anti-glutathionylated protein monoclonal antibody (PSSG, Virogen, n = 4). The kinetics of the S-glutathionylation reaction were analyzed using a standard 2 parameter exponential rise to maximum fitting procedure (Sigma Plot 10, SyStat, MA) for time-dependence following GSSG and PABA/NO (C) for GSTπ+/+ (●) and GST-/- MEF (▲). Data represent mean ± variance, p<0.01.

Enhanced S-glutathionylation following oxidative / nitrosative stress in GSTπ +/+ MEFs

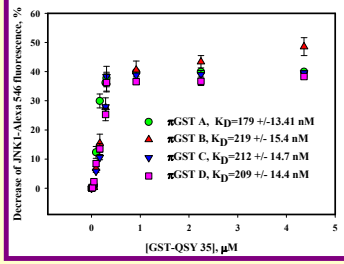


S-glutathionylation of actin is elevated in MEF-GST+/+ cells. MEF-GST+/+ and -/- cells were seeded on glass coverslips and treated with: A - vehicle; B - 300 μM GSSG; C - 15 μM PABA/NO for 1h and stained with phalloidin to visualize actin polymerization/stress fiber formation.



GSTπ expression enhances dose-dependent S-glutathionylation of proteins. Mouse embryo fibroblast cells derived from GSTπ +/+ or (-/-) animals were treated with various concentrations of GSSG (A) or PABA/NO (B) for 1h. Proteins were resolved by non-reducing SDS-PAGE and S-glutathionylation evaluated by immunoblot with PSSG antibody (n=4). The kinetics of the S-glutathionylation reaction were analyzed using a standard 2 parameter exponential rise to maximum fitting procedure (Sigma Plot 10, SyStat, MA) for dose-dependence following GSSG or PABA/NO (C) for GSTπ+/+ (●) and GST-/- MEF (▲). Data represent mean ± variance; p< 0.01.

Polymorphisms of GSTpi and JNK Interactions



Kd analysis: JNK1 was incubated with succinimidylester of Alexa Fluor® carboxylic acid (Invitrogen) and GSTpi A-D were incubated with succinimidylester of QSY® 35 acetic acid (Invitrogen) to label primary amines according to manufacturer's recommendations. The fluorescent analysis of protein binding was performed on a QM-4 spectrofluorometer (PTI). All spectral changes were normalized for JNK1-Alexa 546 fluorescence in PBS (pH=7.4) and presented as % change. The data represent mean±SE for three independent experiments.

Summary

- GSTpi catalyzes the forward reaction of S-glutathionylation
- The catalytic activity is required for S-glutathionylation
- GSTpi A and D have higher catalytic activities and produce more S-glutathionylation following nitrosative stress
- Polymorphisms of GSTpi do not alter its binding to JNK