JAPSC
Journal of Animal and Poultry Sciences, 2016, 5 (4): 42-51
Available online at http://www.JAPSC.com

Biochemical characterization of glutathione S-transferaases purified from chick embryo brain

S. Dasari[1]*, S. Gonuguntla[1], M. Ganjayi[1], B. Meriga[1], and Th. Kedam[1]

[1]*Department of Biochemistry, Sri Venkateswara University, Ttipati 517 502, Andhra Pradesh, India*

Abstract

Glutathione-S-transferases (GSTs) are phase II defence enzymes and they use multi strategies to protect cellular system from both exogenous and endogenous noxious elements. So, researchers and pharmacologists' are showing interest on GSTs from several decades. Generally birds are highly mobile in both earth and air, and they go through various environments polluted by either industrialization or crop system modernized with pesticide, herbicides and etc. But there is no sufficient attention on defence enzymatic system on birds. The present study was aimed to purify chick embryo brain cytosolic GSTs by using glutathione CL-agarose affinity matrices and the purified GSTs were showed significant activities with model substrates. In addition to that, chick embryo brain GST was exhibit glutathione dependent peroxidase activity. Affinity purified chick embryo brain GSTs were resolved into two bands on 12% sodium dodecyl sulphate polyacrylamide gel (SDS PAGE) and they named as CB_I and CB_{II}. Biochemical characterization of GST was agreed with the previous literature. So, it was identified that the affinity purified brain GSTs from chick embryo may be related alpha and mu class.

Key words: Chick embryo barin, Glutathione CL-agarose affinity column, CB_I & CB_{II}, Alpha & Mu GSTs

*Corresponding author: Tel: +91 8978935568;
E-mail address: dasarisreenivaasulu@gmail.com

Introduction

Glutathione S-trasnferses 'EC 2.5.1.1.8' (GSTs) are phase II multifunctional intracellular enzymes which detoxify endogenous and exogenous toxic compounds by conjugation, dehydrochlorination, glutathione peroxidase activity or passive/sacrificial binding (Hayes et al., 2005; Mannervik and Danielson, 1988; Pickett and Lu, 1989; Yang et al., 2001). Cytosolic GSTs are dimeric subunits with molecular weight about 23 to 30 kDa and also possess about 199 to 244 amino acids (Hayes and Pulford, 1995). Usually each subunit contains two domains, one to bind GSH and the other to bind the hydrophobic electrophile (Wang et al., 2009). GSTs are widely expressed in all tissues (Thomson et al., 2004; Fedulova et al., 2010). The key activity of glutathione S-transferase is the conjugation of xenobiotics with glutathione (Satheesh et al., 2010; Oakley, 2011). Glutathione S-transferase (GST) concentration is inversely proportional to cytotoxicity (Verma and Leekha, 2016).

Alpha class GSTs shows more activity with cumene hydroperoxide (Mannervik et al., 1985) and mu class GSTs shows more activity with epoxides. In mammals, alpha and mu class GSTs are most diverse, each class have at least four or more distinct genes (Morel et al., 2002). Mammalian GSTs are classified into four classes such as alpha (α), mu (μ), pi (π), and theta (θ) (Hayes and Pulford, 1995) and sigma and kappa class GSTs are also identified (Meyer and Thomas, 1995; Pemble et al., 1996). Based on the primary amino acid sequence that the GSTs are classified, GSTs isoenzymes with more than 40% similarity are arranged in the same class and those with less than 30% similarity are arranged in different class (Hayes and Pulford, 1995). GST enzymes have many functions throughout evolution (da Fonesca et al., 2010). The nature of enzyme evolution facilitates the alternative substrate acceptance, physicochemical properties, and the network of interactions with other proteins which are presenting multidimensional opportunities of Darwinian evolution; even they have certain boundaries (Zhang et al., 2012).

First time, Booth et al. (1961) has partially purified from rat liver an enzyme that catalysing conjugations with glutathione. The cytosolic GSTs are extensively studied by using affinity chromatography gels which play a key role to characterize them (Hayes ad Pulford, 1995). Both the glutathione-agarose and S-hexylglutathione-agarose gels were allowed to elute different GST isoenzymes (Hayes ad pulford, 1995) and 1-chloro-2,4-dinitrobenzene is general substrate for glutathione S-tranferases (Clark et al., 1973). The present study was aimed to biochemical characterization of purified chick embryo brain GST.

Materials and Methods

Chemicals

Reduced glutathione (GSH), phenylmethylsulfonylfluoride (PMSF), and gltathione-CL agarose affinity column was obtained from Genei, Bangalore, India. NADPH, Glutathione reductase (GR), 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-epoxy-3-(p-nitro-phenoxy) propane (EPNP), p-nitrophenyl acetate (p-NPA),

bromosulfophthalein (BSP), p-nitrobenzyl chloride (p-NBC), N,N,N',N'-tetramethylenediamine (TEMED) including dialysis bag (cellulose membrane) were purchased from Sigma Chemical Company, St. Louis, MO, U.S.A. Cumene hydroperoxide (CHP), hydroxymethyl aminomethane (Tris base), Sodium azide, potassium chloride, sodium dodecyl sulfate (SDS), sucrose, Ammonium persulfate (APS), ethylenediaminetetraacetic acid (EDTA), Ciocalteu's Folin phenol reagent, acrylamide, N,N'-methylene-bisacrylamide (Bis) and 2-mercaptoethanol purchaged from SD fine chemicals company, Mumbai, India. Silver nitrate, sodium thiosulphate, sodium carbonate, glycine, glycerol, ethanol, methanol, glacial acetic acid, formaldehyde and all other chemicals were of reagent grade obtained from local chemical sources.

Incubation and maintenance of eggs and brain tissue collection

Fertilized eggs of Babcock strain chicken were obtained from poultry department, Sri Venkateswara Veterinary University, Tirupati. Eggs about 50 grams weight were maintained by incubation at 37^{o}C with 60% humidity and rotated them for every five hours. On 18th day of incubation, survived embryos were selected for experimentation by using candler light and then brain tissue was collected from them. That collected brain tissue was washed with cold 50 mM Tris HCl buffer (pH 8.0) which contain 1mM EDTA to remove blood and other body fluids. The collected brain tissue was preserved at -20ºC for further experimentation.

Preparation of cytosolic fraction

That the frozen brain tissue was allowed to thaw and then minced them with scissors. In 50 mM Tris-HCl (pH 8.0) buffer containing 0.25M sucrose and 1mM phenylmethanesulphonyl fluoride (PMSF), twenty percent homogenate was prepared in a potter Elvijhem homogenizer which is in ice jacket. Froth and floating lipid materials in the homogenate were removed through filter by using cheese cloth and the resulting homogenate was then centrifuged for two times at 4^{o}C by 45 minutes at 10,000 rpm. That the collected supernatant was termed as cytosolic fraction and it was used as the enzyme source for GST purification and specific activity studies.

Purification of GSTs

Glutathione-CL-agarose affinity column (size about 0.7 x 2.5 cm) which previously equilibrated with 50mM Tris-HCl buffer (pH 8.0) was loaded with dialyzed cytosolic fraction which consider as GST source and then affinity matrices was washed with the same buffer to saturate the matrices with enzyme and then column washed with 50mM Tris-HCl buffer (pH 8.0) containing 0.2M KCl to remove nonspecific proteins from column, till the optical density (O.D) reached to 0.005 at 280 nm. The GST was eluted from the affinity column into 5 ml aliquots with 50mM Tris HCl (pH 8.0) buffer containing 5mM reduced glutathione (GSH), till the O.D reached to 0.005 at 280 nm. GST activity was measured by the method of Habig et al. (1974) and protein content was estimated by the method Lowry et al. (1951), in all active fractions. All the active

fractions were pooled and then subjected to dialysis against 25 mM Tris-HCl buffer (pH 8.0) and finally centrifuged to remove dead proteins and then the purified GST protein was freeze dried by using sucrose. All Purification steps were carried out at 4°C.

Protein Determinations

Cytosolic fraction and affinity purified fraction protein content was estimated by the method of Lowry et al. (1951).

Activity of glutathione S-transferases (GSTs)

Cytosolic fraction and affinity purified fraction GST activity was measured by UV-spectrophotometer with the substrate 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm by the method of Habig et al. (1974).

Substrate Specificity studies

In this study, to screen the affinity purified chick embryo brain GST isozymes, the following model substrates such as 1,2-epoxy-3-(p-nitro-phenoxy) propane (EPNP), p-nitrophenyl acetate (p-NPA), bromosulfophthalein (BSP), p-nitrobenzyl chloride (p-NBC) and cumene hydroperoxide (CHP) were used, in addition to 1-chloro-2,4-dinitrobenzene (CDNB).

GST activities with p-nitrobenzyl chloride,1,2-epoxy-3-(p-nitro-phenoxy) propane, bromosulfophthalein and p-nitrophenyl acetate (PNPA) were determined as described by the method Habig et al. (1974) and GST peroxidase activity was determined as described by the method of Wendel (1981), using cumene hydroperoxide as substrate.

SDS-Polyacrylamide Gel Electrophoresis

Polyacrylamide slab gel electrophoresis was performed with 7.5 % stacking gel and 12 % separating gel by using discontinuous buffer system as described by Laemmli (1970).

Silver Staining

Polyacrylamide gel was silver stained by the method of Blum et al. (1987).

Table 1. A summary of substrate specifies with model substrates

Substrate	Reagent mixture	Extinction coefficient	Absorbance	Method
EPNP	125 mM KH_2PO_4 - pH 6.5,1mM EPNP, 5 mM GSH, Total Vol. 3ml (enzyme 150-300µg)	$0.5x10^3$ cm^{-1}	360 λ	
PNPA	125 mM KH_2PO_4 - pH 7.0,0.3 mM pNPA, 0.5 mM GSH, Total Vol. 3 ml (Enzyme 150-300µg)	$8.79x10^3$ cm^{-1}	400 λ	
BSP	125 mM KH2PO4 - pH 7.5, 1mM BSP, 5 mM GSH, Total Vol. 3ml (enzyme150-300µg)	$4.5x10^3$ cm^{-1}	330 λ	Habig et al. (1974)
PNBC	1005 mM KH2PO4 - pH 6.5,1mM substrate, 5 mM GSH, Total Vol. 3ml (enzyme 50-100µg)	$1.9x10^3$ cm-1	310 λ	
CHP	1.8 ml assay buffer (50 mM Na_2PO_4 (pH 7), 2.5 mM EDTA, 2.5 mM NADPH), GSH 100 l & 100 l GR, (initiator 100 l CHP).Tatal Vol. 3ml (Enzyme 250 µg)	6.32×10^3 cm^{-1}	340 λ	Wendel (1981)

Table 2. Chick embryo brain glutathione S-transferases purification profile

Purification profile	Total protein	Total activity	Specific activiy	Percentage of yield	Purification fold
Cytosolic fraction	34.81	468.02	13.44	100	1.00
Affinity purified GST sample	19.98	163.10	8.16	34.85	0.60

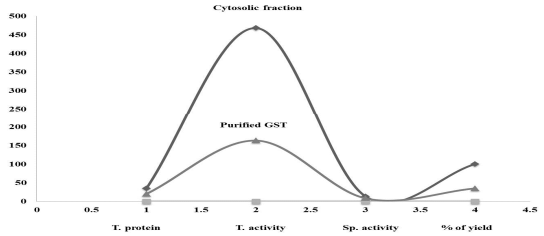

Figure 1. Chick embryo brain cytosolic fraction and purified GST fraction profile

Results

As shown in Table 2, it was calculated that the chick embryo brain cytosolic glutathione S-transferses (GST) specific activity and percentage of yield as 8.16 µmoles/min/mg protein and 34.85%, respectively.

As shown in Fig 1, purified GST fraction shows below half of total activity than crude cytosolic fraction. This is clearly shows certain portion of GST was not bind to the glutathione affinity matrices. This is because which GSTs have high affinity with glutathione CL-agarose matrices was only eluted.

As shown in Fig 2, chick embryo brain purified glutathione S-transferases were showed 81% activity with the substrate CDNB, 26.5% activity with the substrate EPNP, 29.1% activity with the substrate pNPA, 40.5% activity with the substrate BSP, 35.1% activity with the substrate PNBC and 39.7% activity with the substrate CHP.

Table 3. Affinity purified chick embryo brain GSTs specific activity

Model substraates	GSTs specific activiy
1-chloro-2,4-dinitrobenzene (CDNB)	2.98 ± 0.18
1,2-epoxy-3-(p-nitro-phenoxy) propane (EPNP)	0.93 ± 0.14
p-nitrophenyl acetate (PNPA)	1.02 ± 0.06
bromosulfophthalein (BSP)	1.42 ± 0.13
p-nitrobenzyl chloride (pNBC)	1.23 ± 0.72
Cumene hydroperoxide (CHP)	1.39 ± 0.11

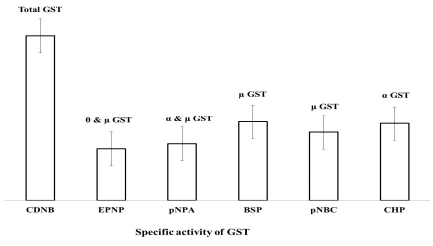

Figure 2. Specific activity of GST isozymes with model substrates.

As shown in Fig 3A, affinity purified chick embryo brain GSTs were showed maximum activity with CDNB and that the GST activity was linearly decreased towards EPNP. But with slightly increased with p-NPA, BSP and slightly decreased with pNBC and slightly increased with CHP. This is because total GSTs in the affinity purified fraction were activity with CDNB. But in case of other substrates, GSTs in affinity purified fraction were showed activity with their specific substrates.

As shown in Fig 3B, substrate CDNB was occupied the first largest space, substrates BSP and CHP were occupied second largest space, substrate pNBC was occupied the third largest space, substrate pNPA was occupied fourth largest space and substrate EPNP was occupied least space with chick embryo brain glutathione S-transferases.

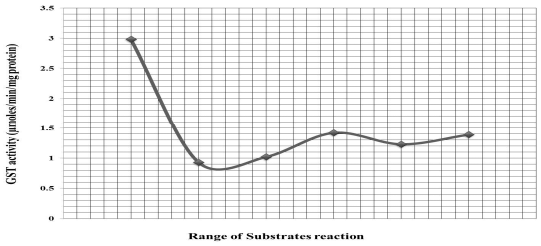

Figure 3A. Range of model substrate reaction with affinity purified GSTs.

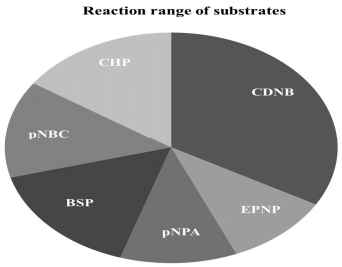

Figure 3B. Range of model substrates reaction with affinity purified GST.

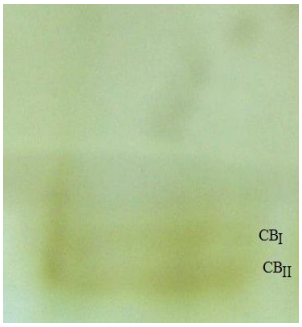

Figure 4. Affinity purified GSTs in 12% SDS PAGE gel visualized by silver staining

Affinity purified chick embryo brain cytosolic glutathione S-transferses were resolved in to two bands on 12% SDS PAGE gel and visualized by silver staining. As shown in Fig 4, they were designated as CB_I and CB_{II}.

Statistical studies

The data related to this study were calculated from three experiments and presented as the mean ± standard deviation (SD).

Discussion

In this study chick embryo brain cytosolic glutathione S-transferase were purified by using glutathione CL-agarose affinity chromatography. Concentrated pooled active fractions were used for both biochemical characterization and SDS PAGE analysis. In this present study, as shown in Fig 3A and B, substrate CDNB was occupied the first largest space, substrates BSP and CHP were occupied second largest space, substrate pNBC was occupied the third largest space, substrate p-NPA was occupied fourth largest space and substrate EPNP was occupied least space with chick embryo brain glutathione S-transferases.

There are several factors (e.g excessive amount of the enzyme loaded to the column, column age and inactivation of enzyme in column) were associated for the low recovery rates of GST (Yu et al., 1989). That the variation in specific activity and protein percentage may be depends on many factors like biological species, isolation, purification and protein quantification methods (Rouimi et al., 1996).

As shown in Fig 1, certain portion of GST was not bound to the glutathione affinity matrices. This is because which GSTs have high affinity with glutathione CL-agarose matrices was only eluted. It was calculated, that the 72-81% of GST activity was retained by glutathione CL-agarose affinity column because certain portion of GST was not bind to the affinity matrices. The present study was agreed with Yu et al. (1989), Rouimi et al. (1996).

Affinity matrices such as glutathione-agarose and S-hyxylglutathione-agarose are widely using to purify alpha (α), mu (μ), pi (π) and sigma (σ) class GSTs because they shows maximum specificity and yield (Hayes and Pulford, 1995). Theta GST purified by neither glutathione-agarose nor S-hexyl-glutathione-agarose but they can purified by affinity chromatography with traizinyl dye gels, Orange A matrix and blue sepharose (Hayes and Pulford, 1995). Both rat testes and brain GSTs were purified by using glutathione-CL agarose affinity column. That purified GSTs specific activity was calculated as 6.5 and 8.6 μmol/min/mg protein, for testes and brain, respectively and percentage of yield was 39% and 32% for testes and brain, respectively (Thyagaraju et al., 2005). In the present study, chick embryo brain glutathione-*S*-transferases were purified according to Thyagaraju et al. (2005) and that the purified GSTs were showed 8.16 mol/min/mg protein of specific activity with the substrate CDNB and percentage of yield was 34.85%.

Coombes and Stakelum (1961), Habig et al. (1974) and Mannervik and Jensson (1982) are said that the mu (μ) GSTs catalyse the conjugation of GSH with BSP in addition to CDNB. Hayes and Pulford in 1995 said that the mu (μ) GSTs are shows significant activity with EPNP. GST catalyses the conjugation of pNPA with GSH through thiolysis and release p-nitrophenol (Satoh et al., 1985) and alpha (α) and mu (μ) GSTs are shows activity with pNPA (Hayes and Pulford, 1995). Mu GSTs shows significant activity with pNBC (Hayes and pulord, 1995). Alpha GST was catalysing the reduction of GSH with CHP (Sherratt and Hayes, 2001).

As shown in Table 3, chick embryo brain purified glutathione S-transferases were showed 85.1% activity with the substrate CDNB, 26.5% activity with the substrate EPNP, 29.1% activity with the substrate pNPA, 40.5% activity with the substrate BSP, 35.1% activity with the substrate pNBC and 39.7% activity with the substrate CHP. So, the present study results were agreed with Coombes and Stakelum (1961), Habig et al. (1974), Mannervik and Jensson (1982), Satoh et al. (1985), Hayes and Pulford (1995), Sherrett and Hayes (2001).

A single substrate can gives activity to another class in addition to its specific class (Mannervik and Danielson, 1988). Rat brain GST isoenzymes showed significant specific activity with CDNB, p-NPA and CHP (Thyagaraju et al., 2005). Wild type alpha GSTs activity increased with pNPA and CHP in addition to CDNB than mutant alpha GST (Zhang et al., 2012). Mu GST activity was enhanced with pNPA and EPNP than genetic variants (Kurtovic et al., 2007).

As shown in Fig 1, chick embryo brain purified glutathione S-transferases were showed 85.1% activity with the substrate CDNB, 29.1% activity with the substrate pNPA, 40.5% activity with the substrate BSP and 39.7% activity with the substrate CHP and 26.5% activity with the substrate EPNP. So, the present study results were agreed with Mannervik and Danielson (1988), Thyagaraju et al. (2005), Zhang et al. (2012) and Kurtovic et al. (2007).

As shown in Fig 4, chick embryo brain glutathione S-transferases purified by glutathione CL-agarose affinity column were named as CB_I and CB_{II}. In addition to that, mu GST shows significant activity with EPNP, p-NPA, BSP and pNBC (Hayes and Pulford, 1995; Mannervik and Jensson, 1982) and alpha GST shows significant activity with p-NPA and CHP (Hayes and Pulford, 1995; Sherratt and Hayes, 2001). Both alpha GST and Mu GST are present in brain as well as in other organs (Mclellan et al., 1992; Mitchell et al., 1997). Thereby the present study results were agreed with Hayes and Pulford (1995), Mannervik and Jensson (1982), Sherratt and Hayes (2001), Mclellan et al. (1992), Mitchell et al. (1997).

Conclusion

In the present study, affinity purified GSTs were showed significant specific activity with certain substrates. Affinity purified chick embryo brain sample activity with BSP and pNBC showed the existence

of mu (μ) class GST and activity with EPNP and p-NPA showed the existence of both alpha (α) and mu (μ) class GSTs. Affinity purified chick embryo brain sample activity towards CHP showed the existence of glutathione dependant peroxidase activity of alpha (α) class GST. In addition to that, that the resolved affinity purified GSTs on 12% SDS PAGE gel named as CB_I and CB_{II}. Both GST activity with model substrates and SDS PAGE gel analysis were showed presence of two classes of GSTs in the purified sample. It was concluded that the affinity purified chick embryo brain sample contains both alpha and mu class GSTs. Based on the model substrates specific reaction with affinity purified sample, CB_I and CB_{II} GSTs are related to alpha and mu class GSTs. Either CB_I or CB_{II} GSTs possess glutathione dependant peroxidase activity.

Conflict of interest

Authors do not have any potential conflict of interest regarding the publisher's policy requirements.

Acknowledgments

I am enormously grateful to the University Grants Commission (UGC), New Delhi, who given generous financial assistance as JRF and SRF (ROC. No.20601/UGC-1/2/RGNF/2007).

References

Hayes, J. D., J. U. Flanagan, and I. R. Jowsey. 2005. Glutathione transferases. *Annual Review of* P*harmacology and Toxicology,* 45: 51–88.

Mannervik, B., and U. H. Danielson. 1988. Glutathione transferases--structure and catalytic activity. *CRC Critical Reviews in Biochemistry,* 23 (3): 283–337.

Pickett, C.B., and A.Y. Lu. 1989. Glutathione S-transferases: gene structure, regulation, and biological function. *Annual Review of Biochemistry*, 58:743-764.

Yang, Y., J.Z. Cheng, S. S. Singhal, M. Saini, U. Pandya, and S. Awasthi. 2001. Role of glutathione S-transferases in protection against lipid peroxidation. *Journal of Biological Chemistry,* 276 (22): 19220-19230.

Hayes, J. D., and J. D. Pulford. 1995. The glutathione S-transferase supergene family: Regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Critcal Reviews of Biochemistry and Molecular Biology,* 30 (6): 445–600.

Wang, X. Y., Z. R. Zhang, and S. Perrett. 2009. Characterization of the activity and folding of the glutathione transferase from Escherichia coli and the roles of residues Cys10 and His106. *Biochemical Journal,* 417 (1): 55–64.

Thomson, R. E., A. L. Bigley, J. R. Foster, I. R. Jowsey, C. R. Elcombe, T. C. Orton, and J. D. Hayes. 2004. Tissue-specific expression and subcellular distribution of murine glutathione S-transferase class kappa. *Journal of Histochemistry and Cytochemistry,* 52 (5): 653–662.

Fedulova, N., F. R. Mathieu, and B. Mannervik. 2011. Characterization of porcine Alpha-class glutathione transferase A1-1. *Archives of Biochemistry and Biophysics,* 507: 205–211.

Satheesh, P., A.A. Rao, G. R. Sridhar, K. Srinivas, and C.S. Akula. 2010. Sequence analysis and homology modeling Gallus gallus glutathione s-transferase (Q08392). *Advances in Experimental Medicine and Biology,* 680: 405-410.

Oakley, A. J. 2011. Glutathione transferases: a structural perspective. *Drug Metabolism Reviews,* 43 (2), 138-151.

Verma, A. K., and A. Leekha. 2016. Elucidation of Glutathione-S-transferase Activity Induced by Pectin-Cisplatin Nano-conjugates for Optimization of New Therapeutic Strategies. *Journal of Nanomedicine and Nanotechnology,* 7 (4): 391-399

Mannervik, B., A. Ê. P. lin, C. Guthenberg, H. Jensson, M. K. Tahir, M. Warholm, and H. Jornvall. 1985. Identification of three classes of cytosolic glutathione transferases common to several mammalian species: correlation between structural data and enzymatic properties. P*roceedings of the National Academy of Sciences, USA.* 82: 7202-7206.

Morel, F., C. Rauch, B. Coles, E. L. Ferrec, and A. Guillouzo. 2002. The human glutathione transferase alpha locus: genomic organization of the gene cluster and functional characterization of the genetic polymorphism in the hGSTA1 promoter. P*harmacogenetics,* 12 (4): 277–286.

Meyer, D. J., and M. Thomas. 1995. Characterization of rat spleen prostaglandin H D-isomerase as a sigma class GSH transferase. *Biochemical Journal,* 311: 739-742.

Pemble, S. E., A. F. Wardle, and J. B.Taylor. 1996. Glutathione S-transferase class kappa: characterization by the cloning of rat mitochondrial GST and identification of a human homologue. *Biochemical Journal,* 319: 749-754.

Da Fonseca, R.R., W. E. Johnson, S. J. O'Brien, V. Vasconcelos, and A. Antunes. 2010. Molecular evolution and the role of oxidative stress in the expansion and functional diversification of cytosolic glutathione transferases. *BMC Evolutionary Biology,* 10 (281): 1-11.

Zhang, W., F. A. R. Daniel Dourado., P. A. Fernandes, M. J. Ramos, and B. Mannervik. 2012. Multidimensional epistasis and fitness landscapes in enzyme evolution. *Biochemical Journal,* 445 (1): 39–46.

Booth, J., E. Boyland, and P. Sims. 1961. An Enzyme from Rat Liver Catalysing Conjugations with Glutathione. *Biochem. J,* 79: 516-524

Clark, A.G., J. N. Smith, and T. W. Speir. 1973. "Cross specificity in some vertebrate and insect glutathione transferases with methyl parathion (dimethyl p-nitro-phenyl phosphorothionate), 1-chloro-2,4-dinitro-benzene and S-crotonyl-N-acetylcysteamine as substrates". *Biochemical Journal,* 135: 385-392.

Habig, W. H., M. J. Pabst, and W. B. Jakoby. 1974. Glutathione S-Transferases. The first enzymatic step in mercapturic acid formation. *The Journal of Biological Chemistry,* 249 (22): 7130-7139.

Lowry, O. H., N. J. Rosenbrough , A.L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry,* 193 (1): 265-275.

Wendel, A. 1981. Glutathione Peroxidase. *Methods in Enzymology,* 77: 325-333

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* 227: 680-685.

Blum, H., H. Beier, and H. J. Gross. 1987. Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. *Electrophoresis,* 8 (2): 93-97.

Yu, S. J. 1989. Purification and characterization of glutathione S-transferases from five phytophagous Lepidoptera. P*esticide Biochemistry and* P*hysiology*, 35: 97-105.

Rouimi, P., P. Anglade, L. Debrauwer, and J. Tulliez. 1996. Characterization of pig liver glutathione S-transferases using HPLC-electrospray-ionization mass spectrometry. *Biochemical Journal*, 317 (Pt3): 879-884.

Thyagaraju, K., B. Hemavathi, K. Vasundhara, A. D. Rao, and K. N. Devi. 2005. Comparative study on glutathione transferases of rat brain and testis under the stress of phenobarbitol and beta-methylcholanthrene. *Journal of Zhejiang University Science B*, 6 (8): 759-769.

Coombes, B., and G. S. Stakelum. 1961. A liver enzyme that conjugates sulfobromaphthalien with glutathione. *Journal of Clinical Investigation*, 40: 981-988.

Mannervik, B., and H. Jensson, 1982. "Binary combination of four protein subunits with different catalytic specificities explain the relationship between six basic glutathione S-transferases in rat liver cytosol". *Journal of Biological Chemistry*, 257: 9909-9912.

Satoh, K., A. Kitahara, Y. Soma, Y. Inaba. I. Hatayama, and K. Sato. Purification, induction and distribution of placental glutathione transferases: 1985. A new marker enzyme for preneoplastic cells in the rat chemical hepatocarcinogenesis. P*roceedings of Natitonal Academy of Sciences (*P*NAS USA) Biochemistry*, (82): 3964–3968.

Sherratt, P. J., and J. D. Hayes. 2001. Pages 319-352 in: Glutathione S-transferases. Enzyme systems that metabolise Drugs and other xenobiotics, John Wely and Sons Ltd. UK.

Mannervik, B., and U. H. Danielson. 1988. Glutathione transferases–structure and catalytic activity. *CRC Critical Reviews in Biochemistry*, 23 (3): 283–337.

McLellan, L. I., D. J. Harrison, and J. D. Hayes. 1992. Modulation of glutathione S-transferase and glutathione peroxidase by the anticarcinogen butylated hydroxyanisole in murine extrahepatic organs. *Carcinogenesis*, 13: 2255-2261.

Mitchell, A. E., D. Morin, J. Lakritz, and A. D. Jones. 1997. Quantitative profiling of tissue- and gender- related expression of glutathione S-transferase isoenzymes in the mouse. *Biochemical Journal*, 325 (1): 207-216.