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Multiple glutathione *S*-transferase isoforms are present on male germ cell plasma membrane

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Abstract Phase II detoxification enzymes, the glutathione *S*-transferases (GSTs) of 24 kDa are known to be cytosolic enzymes. This study shows that multiple GST isoforms that are 24 kDa in size are present on the extracellular side of the plasma membrane of rat male germ cells. The GST activity of male germ cell plasma membranes is several folds higher than somatic cell plasma membrane GST activity. Isoform composition of the germ cell plasma membrane and the cytosolic pool differ, GSTM5 and GSTPi being absent on the plasma membranes. The molecular masses of the common isoforms are comparable between the two pools and both pools show GST and glutathione peroxidase activity.

Key words: Glutathione *S*-transferase; Plasma membrane; Germ cell

1. Introduction

It is well known that damage to testicular male germ cells inflicted by toxins, products of abnormal metabolism or reactive oxygen species (ROS) can result in infertility or defective offspring [1]. Therefore, protection from stress generated by the above agents is of utmost importance to the germ cells. These agents can cause more damage to germ cells in comparison to somatic cells because of two reasons. Firstly, the germ cell plasma membrane is rich in polyunsaturated fatty acids that are prone to oxidation [2] and secondly, the intimate association of the germ cells with the phagocytic Sertoli cells that supply structural support, nutrients, growth factors and hormonal signals [3], exposes them to high levels of ROS generated by the Sertoli cells [4]. A good cellular defense system is therefore required to maintain the integrity of the genome, however, the mechanisms of cellular defense in male germ cells are relatively less explored and information on the possible implications of stress on germ cell biochemistry remains incompletely understood.

Products generated by ROS or metabolites of drugs and chemicals that induce stress need to be eliminated from a cell if cell damage is to be prevented. Detoxification of products of oxidative stress and electrophilic compounds is carried out by a group of enzymes called glutathione *S*-transferases (GST; EC 2.5.1.18) that constitute the phase II detoxification system in cells and are encoded by multiple genes in mammals [5]. GST can catalyze the covalent addition of the tripeptide glutathione (GSH) to a structurally diverse set of physiological and xenobiotic electrophiles where the thiolate of GSH acts as the nucleophile and promotes the formation of GSH adducts. GSTs also catalyze Se-independent GSH peroxidase reactions with organic hydroperoxides as substrates where GSH acts as a reductant with concomitant formation of oxidized GSH (GSSG). Although mammalian GSTs are predominantly cytosolic, they have been found in other cellular compartments as well. Membrane-bound microsomal and mitochondrial forms that differ structurally with the cytosolic forms have been isolated and sequenced [5]. Hepatocytes express microsomal GSTs on their plasma membranes that are 17.2 kDa in molecular mass [6]. Mammalian GSTs can be grouped into at least seven classes namely α, μ, π, σ, θ (the cytosolic forms) [5], the κ (the mitochondrial form) [7] and the microsomal form [5].

GSTs are expressed in a tissue specific manner and multiple isoforms are often found in a single tissue or cell type [5]. Testis is a rich source of GSTs and multiple forms have been isolated from the organ [8]. Our earlier studies show that the seminiferous tubular fluid contains multiple isoforms of GSTs secreted by the Sertoli cells [9,10] and two isoforms are present on sperm where they serve as detoxification enzymes [11,12]. Recently, we have shown that in testicular germ cells, GSTs accord protection against products of oxidative stress that can reduce the rate of oxidative stress-induced apoptosis [13]. As a first step to understand the relevance of germ cell GSTs in the context of high vulnerability of these cells to stress, it is necessary to investigate the sub-cellular distribution of the enzymes and their transferase and peroxidase activities. The major objective of the present study was therefore to investigate the sub-cellular distribution of these enzymes, isoform composition at major locations within the cell and the kinetics of the GST pools.

This report demonstrates that multiple GST isoforms are present on the germ cell plasma membrane that are 24 kDa in size. Interestingly, the plasma membrane-associated GST activity of germ cells is several folds higher as compared to

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Abbreviations: GST, glutathione *S*-transferase; GSH, glutathione; ROS, reactive oxygen species; STM, sucrose–Tris medium; PMSF, phenylmethylsulfonyl fluoride; PBS-CM, phosphate-buffered saline–calcium, magnesium; HBSS, Hanks' balanced salt solution; RP-HPLC, reverse phase-high performance liquid chromatography; CDNB, 1-chloro-2,4-dinitrobenzene; GRD, glutathione reductase; GSSG, oxidized glutathione; *S*-HGSH, *S*-hexylglutathione; TFA, trifluoroacetic acid

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somatic cell plasma membrane activity. The GST isoform composition of the plasma membrane and cytosol differ but both pools demonstrate GSH transferase and peroxidase activity.

2. Materials and methods

Wistar rats (*Rattus rattus*) were obtained from the small animal facility of the National Institute of Immunology (New Delhi, India). GSH Sepharose-4B was obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). Bicinchoninic acid protein assay reagents A and B were purchased from Pierce Chemical Company (Rockford, IL, USA). All other chemicals were purchased from Sigma (St. Louis, MO, USA).

2.1. Preparation of cells

Testicular germ cells were prepared according to Meistrich et al. [14] with modifications as described earlier [13]. Rat brains were digested with 0.25% trypsin and resulting cell suspension was washed and harvested [15]. Rat liver cells were prepared according to Hubbard et al. [16] with minor modifications. Briefly, the tissue was finely chopped in HBSS (Hanks' balanced salt solution) containing 3 mg/ml collagenase/dispase and digested in a gyratory water bath at 200 rpm and 37°C for 30 min. Splenocytes were prepared according to Abraham et al. [17] where spleen were crushed between two frosted slides, suspended in 0.25 M sucrose–Tris medium (STM) (0.25 M sucrose, 10 mM Tris–HCl, 1 mM EDTA) and filtered through cheese cloth. The resulting cell suspension was centrifuged at $500\times g$ and cells harvested. Viability of all cell preparations was checked by 0.1% trypan blue dye exclusion test.

2.2. Preparation of antisera

Antisera against N-terminal peptides designed from N-terminal sequences of GST α, μ and π were raised and characterized as described previously [12].

2.3. Preparation of sub-cellular fractions

Plasma membranes were prepared from germ cells according to Graham et al. [18]. Briefly, homogenates from all the cell types mentioned above were prepared in 0.25 M STM with protease inhibitors (1 mM each of phenylmethylsulfonyl fluoride (PMSF), EGTA and EDTA) and centrifuged at $1000\times g$ at 4°C for 10 min. The supernatant was centrifuged at $15\,000\times g$ in a Beckmann Optima™ XL-100K ultracentrifuge with a SW55Ti rotor at 4°C for 20 min. The supernatant (S-15) of this spin was centrifuged at $20\,000\times g$ at 4°C to obtain the crude mitochondrial fraction. The recovered supernatant was centrifuged similarly as above. The supernatant was used as the cytosol and the pellet was washed with 10 mM Tris–HCl before being loaded onto an 18% (v/v) linear gradient of Percoll to recover membrane fractions. The fractions thus obtained were assayed for 5′-nucleotidase as a marker for plasma membrane purity using ascorbic acid and ammonium molybdate and read for change in absorbance with a spectrophotometer at 820 nm.

2.4. Surface localization of GSTs

Cell surface biotinylation was carried out according to Zurzolo et al. [19]. In brief, 10^8 cells were washed with phosphate-buffered saline–calcium, magnesium (PBS-CM) (50 mM sodium phosphate buffer, 0.14 M NaCl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$) and suspended in the same buffer containing 0.25 mg/ml sulfo-NHS-biotin at 4°C for 30 min. Reaction was quenched by removing the biotin solution and washing twice with cold HBSS. Total GSTs were purified from these cells by GSH Sepharose-4B affinity chromatography, separated on reverse phase-high performance liquid chromatography (RP-HPLC), run on SDS–PAGE (12%) as described previously, transferred onto nitrocellulose and probed with avidin–HRP (1:5000).

For immunofluorescence, live germ cells were stained at 4°C with primary (anti-μN, anti-πN and anti-αN) and secondary antibodies at 1:100 and 1:250 dilutions respectively and all washes were carried out in the cold. For flow cytometry, propidium iodide (1 μg/ml) was added to identify live cells and analysis done as described previously [15] on a Coulter® EPICS® ELITE ESP Flow Cytometer (Beckman Coulter, Inc., Fullerton, CA, USA). Fluorescence data for 10 000 cells were collected with logarithmic amplification for green fluorescence. Confocal images of stained cells were collected with a Zeiss LSM-510 confocal microscope after illumination with 488-nm excitation light from an argon ion laser at 13% power. Fluorescence was collected with a 500–550 band pass filter with pinhole set at 100 μm. For fluorescence microscopy with an optical microscope, epifluorescent illumination was used with a Nikon Optiphot fluorescence microscope.

2.5. Purification of GSTs

GSTs were purified from plasma membrane and cytosolic fractions of germ cells as described previously [13] and electrophoresis was carried out using the buffer system of Laemmli on 12% polyacrylamide gels [20]. GST isoforms were separated by RP-HPLC (Waters 625 LC system, Millipore, Bedford, MA, USA) using a VYDAC C_4 column (3.9 mm × 30 cm) equilibrated with 60% (v/v) solvent 1 (0.08% trifluoroacetic acid (TFA) in water) and 40% (v/v) solvent 2 (0.08% TFA in 80% acetonitrile) as described previously [13].

2.6. Electron spray mass spectrometry (ES-MS)

ES-MS was performed with fractions containing the peaks eluted out from RP-HPLC separation as described earlier [10]. Mathematical transformations of elecrospray spectra to true mass scale were attained with Max Ent algorithm (Fisons Masslynx software), with 1 Da per point.

2.7. GST and GSH peroxidase assay

GST assay was carried out using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate as described previously [13]. Analysis of inhibition of enzyme activity was carried out in the presence of GST inhibitors (*S*-hexylglutathione (*S*-HGSH) and ethacrynic acid) at varying concentrations. Peroxidase activity of GST was measured with cumene hydroperoxide by the coupled enzyme method utilizing excess GSH reductase (GRD), which couples oxidation of NADPH to the reduction of GSSG formed by the Se-independent peroxidase action of GSTs [13]. The reaction mixture contained 1.25 IU/ml of GRD, 1 mM GSH, appropriate amount of affinity-purified germ cell GST, and 1 mM cumene hydroperoxide in 0.1 M sodium phosphate buffer, pH 7.5. The oxidation of NADPH was monitored by the absorbance decrease at 340 nm using a UV-160A UV–visible spectrophotometer (Shimadzu, Tokyo, Japan).

2.8. Statistical analyses

An unpaired two-tailed Student's *t*-test using T-EASE software, Version 2.0 (Institute for Scientific Information®, USA) and ANOVA were used for statistical analyses.

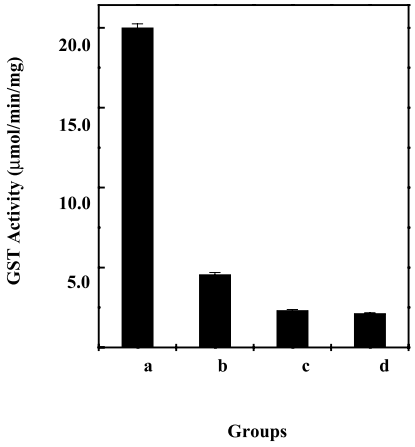

Fig. 1. Plasma membrane-associated GST activities in somatic and germ cells. Purified plasma membranes from germ cells, and cells of the liver, brain and spleen were assayed for GST activity with 1 mM CDNB and 1 mM GSH. a: Plasma membranes from germ cells; b: plasma membranes from liver cells; c: plasma membranes from brain cells; d: plasma membranes from spleen cells. Results are mean ± S.D. of four repeats. Each repeat consisted of a pool of cells from six rats.

Table 1
ES-MS analysis of rat germ cell cytosol and plasma membrane GSTs

Peak 1	GST isoform	Testicular GST	Cyt-GST	PM-GST
Peak 1	rGSTM1	25 784 ± 2.00	25 764 ± 2.63	25 766 ± 9.34
Peak 2	rGSTM2	25 572 ± 1.7	25 572 ± 0.89	25 574 ± 8.01
Peak 3	rGSTPi	23 310 ± 3.7	23 309 ± 7.33	Absent
Peak 4	rGSTA3	25 190 ± 3.7	25 715 ± 10.00	25 202 ± 4.33
Peak 5	rGSTM3	25 550 ± 2.3	25 548 ± 3.99	25 551 ± 5.60
Peak 6	rGSTM5	26 541 ± 2.3	26 541 ± 0.78	Absent

Masses of RP-HPLC-separated affinity-purified cytosol and plasma membrane GSTs as determined by ES-MS. Cyt-GST, GST from rat germ cell cytosol; PM-GST, GSTs from rat germ cell plasma membrane.

3. Results

3.1. Sub-cellular distribution of GSTs and isoform composition

Investigations into the sub-cellular distribution of GST activity in germ cells using an universal GST substrate CDNB revealed that there was no detectable activity in the nuclear fraction while 75% activity was confined to the cytosol. Plasma membrane, mitochondria and microsomal fraction showed 10, 6 and 3.8% of the total cellular activity respectively. The purity of the plasma membrane fraction was ensured by 5′-nucleotidase assay of the different fractions. Although the plasma membrane of germ cells contained only 10% of the total cellular GST activity, the comparative activity with plasma membrane preparations from other cell types showed that germ cell plasma membrane GST activity was several folds higher than that of any other cell type (Fig. 1). GSTs have been primarily described as cytosolic enzymes barring one report where plasma membrane-associated GSTs of molecular weight 17.2 kDa were reported on hepatocytes [6]. In our study, the hepatocyte plasma membrane GST activity was 4-fold less than the germ cell plasma membrane GST activity, the GST activity of plasma membranes from astrocytes and splenocytes being negligible. Because of the fragile nature of the germ cell plasma membrane, the presence of GST activity in the membrane fraction was deemed to be important for further analysis in terms of isoform composition and their function.

Our results demonstrate the presence of multiple GST isoforms of 24 kDa molecular mass on the plasma membrane when GSH affinity-purified GSTs from the plasma membranes were analyzed on SDS–PAGE (Fig. 2A, a and b) and RP-HPLC (Fig. 2B, a and b). Rat GSTM1, rGSTM2, rGSTM3 and rGSTA3 were the primary isoforms. All the above isoforms were present in the cytosol in addition to

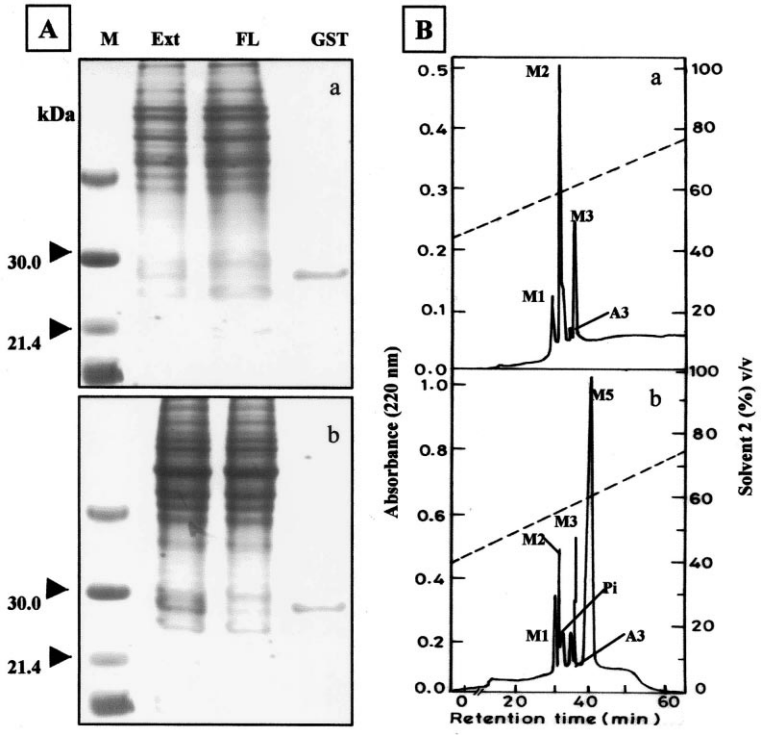

Fig. 2. Purification and analysis of the plasma membrane and cytosolic pool of rat germ cell GSTs. A: SDS–PAGE of plasma membrane (a) and cytosolic GSTs (b) at different stages of purification. M, molecular weight marker; Ext, NP-40 extract of rat germ cells; FL, flow-through of the GSH Sepharose-4B column; and GST, affinity-purified GST eluted from the GSH Sepharose-4B column. B: RP-HPLC profile of rat germ cell plasma membrane and cytosolic GSTs. The affinity-purified rat germ cell GSTs separate into multiple peaks. a: RP-HPLC profile of plasma membrane GSTs showing the presence of rGSTM1, rGSTM2, rGSTA3 and, rGSTM3; b: RP-HPLC profile of cytosolic GSTs showing the presence of rGSTM1, rGSTM2, rGSTPi, rGSTA3, rGSTM3 and rGSTM5.

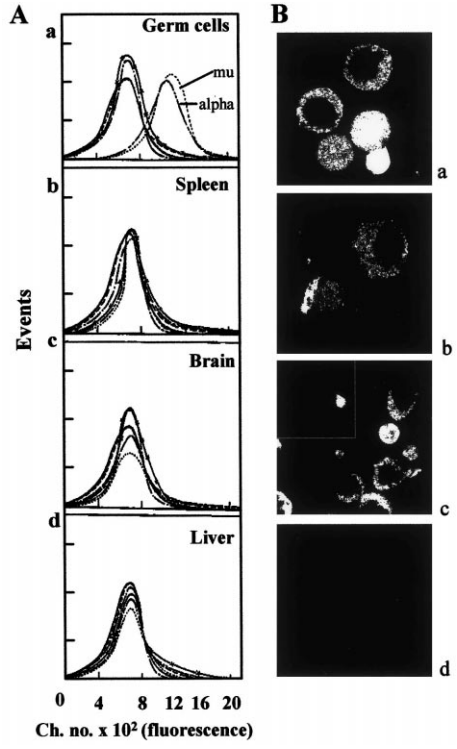

Fig. 3. Surface localization of GSTs on germ cells. A: Flow cytometry data. Cells were stained with class specific anti-GST μN (1:100), anti-GST πN (1:100) and anti-GST α (1:150) antibodies at 4°C followed by staining with anti-rabbit IgG-FITC used at a dilution of 1:250. The FITC positive cells were scored in a Coulter® EPICS® ELITE ESP Flow Cytometer. a: Germ cells show a significant shift with anti-GST α and anti-GST μN antibodies indicating the presence of the two isoforms on the surface of germ cells. b–d: Cells of the spleen, brain and liver did not stain positive with any of the three anti-GST antibodies. Viability of the cells was determined by staining with propidium iodide. B: Immunofluorescence of paraformaldehyde-fixed saponin permeabilized rat germ cells with anti-GST antibodies. Serum containing anti-GST IgG was used at 1:100 dilution followed by anti-rabbit IgG-FITC at a dilution of 1:500. The staining is a representative result of three repeats. a: Germ cells stained with anti-μN; b: germ cells stained with anti-πN; c: germ cells stained with anti-α; d: control cells. Inset in (c) shows anti-α antibody staining the acrosome region of a round spermatid.

having rGSTM5 and rGSTPi, rGSTM5 being most abundant (Fig. 2B, b). Rat GSTM5 has been found to be predominantly expressed in germ cells previously in two separate studies [21,22]. Cytosolic and membrane GSTs were very similar in molecular mass barring cytosolic rGSTA3 that was larger than its plasma membrane counterpart by 513 Da (Table 1). The masses of the cytosolic and plasma membrane GSTs are very similar to the reported masses for the testicular and liver GSTs [22].

3.2. Surface orientation of GSTs

Since it was theoretically possible for cytosolic GSTs to bind to membrane lipids inside the cell as they have a strong affinity for lipid like ligands, studies were undertaken to establish the presence of GSTs on the outer surface of the plasma membrane. A shift in flow cytometric (FACS) profile was observed when live germ cells were stained with anti-GST μ and α antibodies but not with anti GST π antibodies (Fig. 3A, a). Somatic cells did not show any shift in the FACS profile with any of the antibodies indicating either absence of expression of the isoforms or presence of extremely low expression beyond detectable limits of the assay (Fig. 3A, b–d). Permeabilized germ cells stained for both cytoplasmic and membrane-associated GSTs showed localization in the cell cytosol as well as in the acrosomal cap (Fig. 3B, a–d). Controls stained with preimmune serum did not show any staining. To distinguish the staining of GSTs on the surface, staining of germ cells without permeabilization was carried out. In the non-permeabilized cells surface GST staining was confined to the membrane overlying the acrosome at various stages of development of sperm as shown in Fig. 4A–H. Therefore, it can be inferred that the bulk of the plasma membrane-associated GSTs recovered from the germ cells were associated with the membranes overlying the acrosome.

rGSTA3, rGSTM3, rGSTM2 and rGSTM1 recovered from affinity-purified GSTs from the surface biotinylated germ cells by RP-HPLC (Fig. 5A) reacted to avidin–HRP on Western blots showing that they were available on the cell surface (Fig. 5B). Rat GSTM5 and rGSTPi did not react with avidin–HRP (Fig. 5B) indicating they were intracellularly localized. This confirms the data obtained with RP-HPLC separation of the

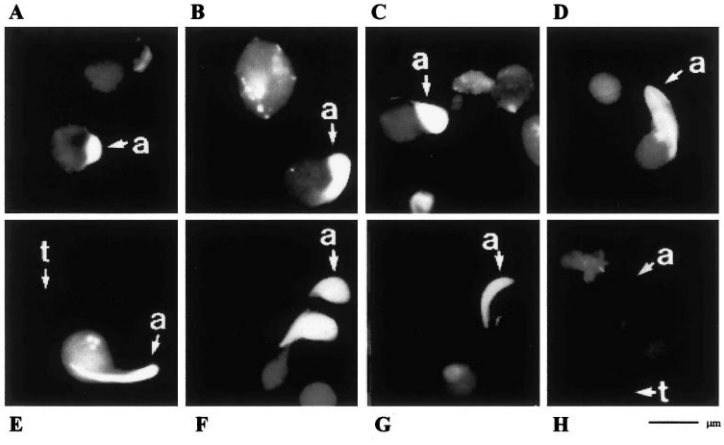

Fig. 4. Localization of GSTs on paraformaldehyde-fixed non-permeabilized germ cells. Rat male germ cells stained with anti-GSTMuN antibodies. Serum containing anti-GSTMu IgG was used at 1:100 dilution followed by anti-rabbit IgG-FITC at a dilution of 1:500. A,B: Staining of round spermatids. C–F: Staining of elongated spermatids, (G) mature testicular sperm, (H) controls of mature testicular sperm stained with preimmune serum. All cells were counterstained with propidium iodide. Similar staining pattern was noted with anti-GST α antibodies. a: acrosome; t: tails.

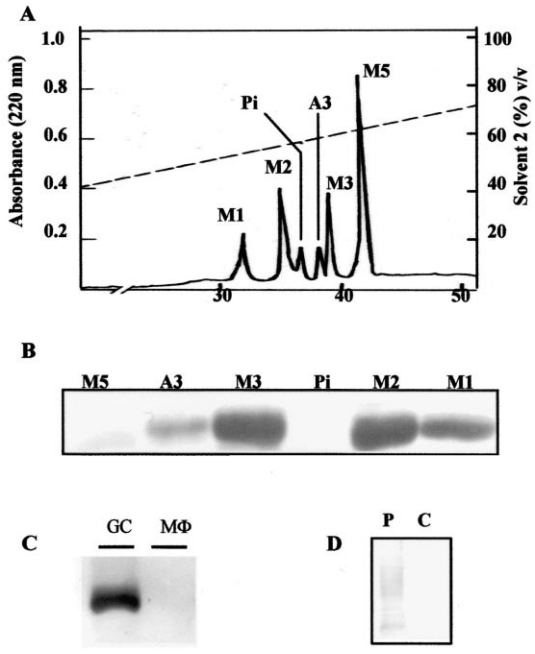

Fig. 5. Biotinylation of rat germ cells. A: RP-HPLC elution profile. GSH Sepharose affinity-purified GSTs from biotinylated live rat germ cells were separated on an C_{18} RP-HPLC column. The GSTs resolved into six peaks namely rGSTM1, rGSTM2, rGSTPi, rGSTA3, rGSTM3 and rGSTM5. B: Western blot of RP-HPLC-separated peaks of GST from surface biotinylated germ cells probed with avidin–HRP (1:2500). Peaks rGSTM1, rGSTM2, rGSTA3 and rGSTM3 are positive for HRP indicating their presence on the plasma membrane of rat germ cells. C: Western blot of GSTs from biotinylated germ cells and macrophages. The GST pool from germ cells stained positive for biotinylation. GC, germ cells; Mϕ, macrophage. D: Western blot of plasma membrane and cytosol of biotinylated germ cells. The plasma membrane shows positivity for biotinylated proteins. No reaction with the cytosolic proteins indicated absence of biotinylation of internal proteins. P, plasma membrane; C, cytosol.

plasma membrane GSTs showing that only rGSTA3, rGSTM3, rGSTM2 and rGSTM1 but not rGSTM5 and rGSTPi could be recovered. Macrophages, used as control cells, that were biotinylated along with the germ cells did not react to avidin–HRP on Western blots (Fig. 5C,D).

Therefore, the detection of GST isoforms on live germ cells by immunofluorescent staining accompanied by surface biotinylation of live germ cells show that GSTs are localized on the extracellular side of the plasma membrane as well as within the cytosol.

3.3. Enzymatic activities

GSTs exhibit both GSH conjugation and Se-independent GSH peroxidase activities [5,23]. Specific activities and kinetic constants of GSH conjugation and GSH peroxidase activities of cytosol and plasma membrane pool of GSTs were determined using the substrates CDNB and cumene hydroperoxide (Table 2) respectively. The cytosolic pool of GSTs showed higher V_{max} for CDNB than the plasma membrane pool whereas the V_{max} for the two pools was same for cumene hydroperoxide. The plasma membrane pool showed lesser K_m for CDNB than the cytosolic pool.

4. Discussion

The most important finding of this study is the presence of multiple GST isoforms on the plasma membrane of male germ cells. Two earlier studies including one from this laboratory have demonstrated the presence of GST activity in rat germ cells [24,13] while another study on isolated spermatocyte population has indicated insignificant activity [25]. Apart from studies on enzyme activities, one report details the cloning of rGSTM5 from the mouse spermatogenic cells [21]. In addition to these reports, a number of studies have detailed the isoform composition of the testis but have used whole testicular tissue to determine the isoform composition [5]. Since testis contains multiple cell types [26] each with a distinct function and importance of its own, the overview of total testicular GSTs is of limited value as the different cell types may vary in terms of isoform composition of GSTs. An example is the brain where class μ GSTs occur in astrocytes whereas class π GSTs are for the most part found in oligodendrocytes [27]. Similarly, in the rat liver, hepatocytes express class α and μ GSTs whereas cells of bile-duct express class π GSTs [28]. It is noteworthy that in germ cells itself, there is a variation in expression of isoforms in the plasma membrane and the cytosol. Although the class π enzyme is the universally distributed isoenzyme especially in somatic tissues, its absence in germ cell membranes and a relatively minor presence in the cytosol show that rGSTPi is relatively less important in this cell type. This observation is consistent with a study where it was shown that GST α and μ were strongly expressed in all normal human testis, the π isoform could not or barely be detected [5]. We found that the most abundant isoform was rGSTM5 in the germ cell cytosol. It is reported that rGSTM5 is a germ cell specific isoform and levels of this isoenzyme sharply increase at 6–7 weeks of development in the rat [22]. We did not detect rGSTM5 in the seminiferous tubular fluid in our earlier studies [10], but the observation in the present study of the predominant presence of the isoform in the germ cell cytosol shows that rGSTM5 may have a specific function that is still unknown. The absence of rGSTM5 on the plasma membrane may relate to the susceptibility of rGSTM5 to ROS-induced inactivation and hence its presence on the membrane would not be beneficial

Table 2
Kinetic constants of cytosol and plasma membrane pool of GSTs with CDNB and cumene hydroperoxide

Substrate	PM-GST		Cyt-GST	
	K_m (mM)	V_{max} (μmol/min/mg)	K_m (mM)	V_{max} (μmol/min/mg)
CDNB	0.05	92.5	0.112	400.2
CuOOH	2.0	0.693	2.72	0.883

CDNB activity was determined at 1.0 mM CDNB/1.0 mM GSH, 25°C, pH 6.5. For determination of K_m CDNB was taken at a range of 0.25–2.0 mM/1 mM GSH. Cumene hydroperoxide activity was determined at 1 mM CuOOH/1 mM CDNB, 37°C, pH 7.4 Determination of K_m for CuOOH was done at a substrate range of 0.25–1.00 mM CuOOH/1 mM GSH. Specific activities are the means of triplicates. Results are mean ± S.D. CuOOH, cumene hydroperoxide; Cyt-GST, cytosolic GST pool; PM-GST, plasma membrane GST pool.

to the cell. On the other hand the presence of α and μ GSTs in the germ cell membrane may be beneficial in terms of efficiency in the elimination of products of lipid peroxidation [5].

Membrane damage through lipid peroxidation is one of the most destructive effects of ROS [29], and systems that maintain adequate antioxidant levels around membranes are therefore required. In the scenario within the seminiferous tubules where germ cells are constantly exposed to ROS and other metabolites from the Sertoli cells, the presence of GSTs on the plasma membrane is beneficial to the cell in terms of a facilitated method of elimination of the harmful products. The much higher plasma membrane-associated GST activity in the germ cells in comparison to somatic cells confirms the necessity of a membrane-bound detoxification system in germ cells. Unpublished data from this laboratory show that inhibition of plasma membrane GST activity lead to increased membrane damage. Although hepatocytes contain GSTs on their plasma membrane capable of utilizing CDNB as substrate, they differ in their molecular mass (17.2 kDa) from the cytosolic GSTs that are 24 kDa in size [6]. The comparative GST activity between germ cells and hepatocytes in our studies show that GST activity in germ cell plasma membranes is 4-fold higher than the hepatocyte plasma membrane. The fact that with our purification strategy we could not identify the 17.2 kDa liver plasma membrane form may indicate either the absence of the 17.2 kDa form from germ cell plasma membrane or the inability to purify by GSH affinity chromatography. The abundance of GSTs in the germ cell raises another interesting possibility. Our earlier studies have demonstrated the capability of the STF-GSTs to bind to steroids [10], therefore, it is possible that the steroid binding function of these enzymes may be utilized by germ cells as these cells are dependent on steroid hormones.

Recently, we reported increased apoptosis of germ cells in vitro, when GST activity was inhibited and an increase in activity when cells were exposed to oxidative stress thereby implicating GSTs as a part of adaptive response of germ cells to stress [13]. The higher efficiency of the cytosolic pool of GSTs in GSH conjugation activity than the plasma membrane pool as shown by the higher V_{max} for the cytosolic pool may be due to the ready supply of GSH from the intracellular sources. The low K_m of the plasma membrane pool indicates a higher affinity for substrates possibly to ensure efficient detoxification even of small amounts of toxins generated near the membranes thus preventing the initiation of a chain reaction. Since the two pools of GSTs are not one single isoform but are composed of different ratios of GST isoforms, these results should however be interpreted with caution. Nevertheless, it should be kept in mind that in an in vivo situation it is the pool of GSTs that are acting together rather than isolated forms. Though the cytosolic pool differs from the plasma membrane pool in having rGSTM5 and rGSTPi in addition to other isoforms, the two pools still have comparable K_m and V_{max} for cumene hydroperoxide, a reflection on their ability to detoxify substrates by the Se-independent GSH peroxidase activity. It is possible that the Se-independent GSH peroxidase activity possibly is contributed predominantly by rGSTA3, rGSTM1, rGSTM2, and rGSTM3, which are present in comparable amounts in the two pools of GSTs. It has been suggested that some natural substrates for the peroxidase activity of the GSTs include hydroperoxides, lipid epoxides and aldehydes formed from membrane lipids during oxidative stress [5]. This is supported by a study on human GSTs that shows detoxification of lipid epoxides generated during the process of oxidative stress in the lung by the GSH transferase activity of the GSTs [30]. The plasma membrane is very susceptible to damage because of the chain reactions set in by toxins that peroxidize the lipids. Due to this reason, even small amounts of toxins could be more damaging in the vicinity of plasma membranes than when it enters the cytosol.

The orientation of the germ cell GSTs towards the extracellular side as evidenced by surface biotinylation of live germ cells and indirect immunofluorescence studies indicates that in addition to using intracellular GSH, germ cells have the option of using GSH generated by the Sertoli cells or GSH present in the tubular fluid for conjugation of electrophiles formed on the membranes. This gives them more flexibility for protection. It is known that seminiferous tubular fluid contains GSH [31], and under the observations obtained from this study it seems a distinct possibility that the surface GSTs on germ cells are being utilized to ensure extra protection to the membrane that is rich in unsaturated lipids. From nature's point of view, this is very important as it ensures additional protection for this very vital cell type.

In summary, this study clearly establishes the presence of GSTs on the plasma membrane of germ cells that resemble their cytosolic counterparts very closely in molecular mass. The observation that activity of germ cell plasma membrane GST activity is several folds higher than somatic cell plasma membranes indicate the importance of GSTs in the very vulnerable germ cell types.

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