Published in final edited form as:

Chem Res Toxicol. 2007 January ; 20(1): 149–154. doi:10.1021/tx600305y.

Glutathione Transferase Omega 1 Catalyzes the Reduction of S-(Phenacyl)glutathiones to Acetophenones

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Abstract

S-(Phenacyl)glutathione reductase (SPG-R) plays a significant role in the biotransformation of reactive α -haloketones to non-toxic acetophenones. Comparison of the apparent subunit size, amino-acid composition, and catalysis of the reduction of S-(phenacyl)glutathiones indicated that a previously described rat SPG-R (Kitada et al. (1985) *J. Biol. Chem.* 260,11749-11754) is homologous to the omega-class glutathione transferase GSTO1-1. The available data show that the SPG-R reaction is catalyzed by GSTO1-1 and not by other GSTs, including the closely related GSTO2-2 isoenzyme. In the proposed reaction mechanism, the active-site cysteine residue of GSTO1-1 reacts with the S-(phenacyl)glutathione substrate to give an acetophenone and a mixed disulfide with the active-site cysteine; a second thiol substrate (*e.g.*, glutathione or 2-mercaptoethanol) reacts with the active-site disulfide to regenerate the catalytically active enzyme and to form a mixed disulfide. A new spectrophotometric assay was developed that allows the rapid determination of SPG-R activity and specific measurement of GSTO1-1 in the presence of other GSTs. This is the first specific reaction attributed to GSTO1-1, and these results demonstrate the catalytic diversity of GSTO1-1, which, in addition to SPG-R activity, catalyzes the reduction of dehydroascorbate and monomethylarsonate (V) and also possesses thioltransferase and GST activity.

Introduction

The cytosolic glutathione transferases are a large family of enzymes that play significant roles in both the detoxication and bioactivation of xenobiotics and the catabolism or synthesis of a number of important endogenous compounds (1). The recently identified omega-class glutathione transferases (GSTs) are widely distributed across a range of species from *Caenorhabditis elegans* to humans (2,3). Two human omega-class genes have been characterized, and their RNA and protein (GSTO1-1 and GSTO2-2) products have been identified in multiple tissues (4). The omega-class GSTs are of considerable interest because of the discovery of their role in the reduction of methylated arsenic species and their genetic linkage to the age-at-onset of both Alzheimer's and Parkinson's diseases (5-9). An omega-class GST has also been implicated in the processing and release of the proinflammatory cytokine interleukin-1 (10). Both GSTO1-1 and GSTO2-2 have been expressed in *Escherichia coli* and characterized (6). The crystal structure of GSTO1-1 has been determined and revealed an active-site cysteine residue. The presence of an active-site cysteine residue differs significantly from the tyrosine or serine residue found in the active site of other mammalian cytosolic GSTs (2). Both human omega-class GSTs exhibit thioltransferase, dehydroascorbate reductase, and

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monomethylarsonate (V) reductase activities that are dependent on an active-site cysteine residue (3). These reactions are not catalyzed by other GST classes, and the dehydroascorbate reductase activity of GSTO2-2 is high, indicating that this may be its primary physiological function (6). Because of their involvement in novel reduction reactions and the association of the omega-class GSTs with neurological diseases that may be associated with exposure to environmental agents, we have investigated other potential substrates of physiological and toxicological interest.

α -Haloketones are important, biologically active chemicals that have a variety of uses and avenues for human exposure. Some α -haloketones have been identified as metabolites of insecticides (11), and 2-chloroacetophenone is used as a temporary incapacitating agent (tear gas) (12). α -Haloketones are also used in laboratory research as affinity labels and as active-site inhibitors of enzymes (13,14). α -Haloketones react readily with sulfur nucleophiles, and 2-chloroacetophenone depletes glutathione concentrations in isolated rat hepatocytes (15).

Previous studies showed that the reduction of toxic α -haloketones to nontoxic acetophenones requires glutathione (11,16-18). In the initially proposed reaction mechanism, α -haloketones react nonenzymatically with glutathione to yield *S*-(phenacyl)glutathiones, which are the true substrates of an enzyme that was termed *S*-(phenacyl)glutathione reductase (SPG-R) (18,19). The enzyme was thought to catalyze the attack of a thiol on the sulfur atom of the *S*-(phenacyl) glutathione to yield a disulfide and a carbanion that is stabilized by enolization and yields the corresponding acetophenone after protonation (18) (Figure 1). SPG-R has been purified from rat hepatic cytosol and has a molecular weight of 30–37 kDa that is similar to the apparent subunit molecular weight of GSTO1 after SDS-PAGE (2,19). Comparison of the amino acid composition of each enzyme also revealed a strong similarity.

We report herein that GSTO1-1 has significant SPG-R activity and that the active-site Cys₃₂ plays a critical role in the reaction. SPG-R activity was also characterized with a new, selective spectrophotometric assay. The data show that GSTO1-1 is likely homologous to the SPG-R that was previously purified from rat liver (19).

Materials and Methods

Enzyme purification

Human GSTO1-1 was expressed in *E. coli* (M15-rep4) from the plasmid pQE30 (QIAGEN, Hilden, Germany), as previously described (3). This expression vector generates recombinant enzymes with 6 histidine residues at the *N*-terminus to facilitate purification. The enzyme was purified by chromatography on Ni-agarose. After elution with imidazole, the purified enzyme was dialyzed against 20 mM Tris-HCl, 60 mM NaCl, pH 8, and concentrated to 2 mg mL⁻¹ before storage at -20 °C. GSTO2-2 was also expressed in *E. coli* from a pQE30 plasmid. GSTO2-2 is prone to the formation of inclusion bodies in *E. coli* and precipitation during purification. Soluble GSTO2-2 was successfully generated by the use of a low-temperature bacterial expression protocol and the application of a cyclic urea gradient refolding strategy (6).

Instrumental analyses

ESI-MS analyses were performed by direct injection with an Agilent LC/MSD ion-trap mass spectrometer (Agilent Technologies) with an electrospray interface operated in the positive-ion mode: dry temp, 325 °C; nebulizer, 10 psi; drying gas, 4 L min⁻¹, skim, 40.5 V; capillary exit, -3000 V; and trap drive, 32.5. ¹H NMR spectra were recorded with a Bruker 400 MHz spectrometer; samples were dissolved in [2H₆]DMSO, and chemical shifts are reported in ppm downfield from TMS.

Synthesis of *S*-(phenacyl)glutathiones

S-(Phenacyl)glutathiones (Figure 2) were prepared by the method of Vince et al. (20) by reaction of glutathione (Sigma) with 2-chloroacetophenone **1a** (Fluka), 2,2',4'-trichloroacetophenone **2a**, 2-chloro-4'-fluoroacetophenone **3a**, or 3-chloropropiophenone **4a** (Aldrich). The precipitated products were washed extensively with ice-cold water to remove residual glutathione, dried, and stored at -20°C . The removal of residual glutathione was confirmed by the absence of a reaction of the *S*-(phenacyl)glutathiones with 5,5'-dithiobis-(2-nitrobenzoic acid). Before use, the *S*-(phenacyl)glutathiones were solubilized briefly in 1M NaOH before rapid neutralization with Tris-HCl.

***S*-(Phenacyl)glutathione 1b**—ESI-MS, m/z 448 $[\text{M} + \text{Na}]^{+}$, m/z 470 $[\text{M} + 2\text{Na} - \text{H}]^{+}$, m/z 510 $[\text{M} + 3\text{Na} - 2\text{H}]^{+}$. NMR, 1.93 (2H, pentet (apparent), $J = 6.8$ Hz); 2.34 (2H, t, $J = 7.2$ Hz); 2.52 (2H, s); 2.69 (1H, dd, $J = 9.6$ Hz); 2.94 (1H, dd, $J = 13.6$); 3.39 (1H, t, $J = 6.8$ Hz); 3.72 (2H, m); 4.06 (1H, d, $J = 15.2$ Hz); 4.15 (1H, d, $J = 15.2$ Hz); 4.52 (1H, m); 7.54 (1H, t, $J = 7.6$ Hz); 7.66 (1H, t, $J = 7.2$ Hz); 7.99 (1H, d, $J = 7.2$ Hz); 8.46 (1H, d, $J = 8.8$ Hz); 8.62 (1H, t, $J = 5.6$ Hz).

***S*-(2',4'-Dichlorophenacyl)glutathione 2b**—ESI-MS, m/z 516 $[\text{M} + \text{Na}]^{+}$, m/z 538 $[\text{M} + 2\text{Na} - \text{H}]^{+}$, m/z 560 $[\text{M} + 3\text{Na} - 2\text{H}]^{+}$. ^1H NMR, 1.90 (2H, m); 2.32 (2H, m); 2.52 (2H, s); 2.67 (1H, dd, $J = 13.6$ Hz); 2.94 (1H, dd, $J = 13.6$ Hz); 3.4 (obscured by water peak); 3.72 (2H, s); 3.96 (2H, d, $J = 15.6$ Hz); 4.02 (d, $J = 15.6$ Hz); 4.48 (1H, m); 7.58 (1H, d, $J = 6.4$ Hz); 7.78, (1H, m); 8.70 (1H, t, $J = 5.6$ Hz).

***S*-(4-Fluorophenacyl)glutathione 3b**—ESI-MS, m/z 466 $[\text{M} + \text{Na}]^{+}$, m/z 488 $[\text{M} + 2\text{Na} - \text{H}]^{+}$, m/z 510 $[\text{M} + 3\text{Na} - 2\text{H}]^{+}$. ^1H NMR, 1.90 (2H, m); 2.32 (2H, m); 2.68 (1H, dd, $J = 13.6, 9.6$ Hz); 2.94 (1H, dd, $J = 13.6, 4.4$ Hz); 3.34 (1H, t, $J = 7.0$ Hz); 3.72 (2H, m); 4.05 (1H, d, $J = 15.2$ Hz); 4.14 (1H, $J = 15.0$ Hz); 4.50 (1H, m); 7.38 (1H, t, $J = 7.6$ Hz); 8.08 (1H, m); 8.44 (1H, d, $J = 8.4$ Hz); 8.66 (1H, m).

***S*-(2-Benzoyl)ethyl)glutathione 4b**—ESI-MS, m/z 462 $[\text{M} + \text{Na}]^{+}$, m/z 484 $[\text{M} + 2\text{Na} - \text{H}]^{+}$, m/z 506 $[\text{M} + 3\text{Na} - 2\text{H}]^{+}$. ^1H NMR, 1.92 (2H, m); 2.34 (2H, m); 2.68 (1H, dd, $J = 14.0, 9.6$ Hz); 2.83 (1H, t, $J = 7.2$ Hz); 2.83 (1H, t, $J = 7.2$ Hz); 2.96 (1H, dd, $J = 14.0, 4.8$ Hz); 3.36 (1H, m); 3.36 (2H, m); 3.72, (2H, s); 4.48 (1H, m); 7.54 (1H, m); 7.65 (1H, m); 7.99 (1H, $J = 7.6$ Hz); 8.42 (2H, d, $J = 8.4$ Hz); 8.69 (1H, $J = 5.6$ Hz).

Measurement of enzyme activities

Assay I. This spectrophotometric method relies on the glutathione reductase-catalyzed reduction of glutathione disulfide and the concomitant oxidation of NADPH; glutathione disulfide is formed by the GSTO1-1-catalyzed reaction of glutathione with an *S*-(phenacyl) glutathione. The reaction mixtures contained 1 mM 2,2',4'-trichloroacetophenone **2a**, 10 mM glutathione, 0.3 mM NADPH, 1.5 mM EDTA acid, 1 unit of glutathione reductase (Sigma), 100 mM potassium phosphate buffer, pH 7.4, and purified recombinant GSTO1-1 or GSTO2-2 in a final volume of 1 mL. The reaction mixture was incubated at 37°C , and the rate of oxidation of NADPH was recorded at 340 nm. A mM absorptivity of 6.22 was used to calculate the reaction rate, which is expressed as $\mu\text{mol min}^{-1} \text{mg protein}^{-1}$. In some experiments, other α -haloketones were used as substrates. Control reaction mixtures lacked GSTO1-1 or GSTO2-2.

Assay II. This spectrophotometric method records the reduction of the *S*-(phenacyl)glutathione substrate directly. Typically, the reaction mixtures contained 100 mM Tris, pH 8.0, 1.5 mM EDTA, 10 mM 2-mercaptoethanol or glutathione, 0.5 mM *S*-(phenacyl)glutathione, and purified recombinant GSTO1-1 or GSTO2-2. The reaction mixtures were incubated at 37°C , and the absorbance was recorded from 250 to 400 nm. The wavelength of the maximum

absorbance change for each *S*-(phenacyl)glutathione substrate was determined by inspection of the recorded spectra (Figure 3). A mM absorptivity was calculated by relating the absorbance change to the concentration of substrate determined independently by allowing Assay I to proceed to completion in the presence of GSTO1-1. The mM absorptivity represents the absorbance change of a 1mM solution of substrate with a 1cm light path. The wavelength of maximum absorbance and the mM absorptivities for each substrate were found to be; *S*-(Phenacyl)glutathione **1b**, 271nm, -1.89 OD units; *S*-(2',4'-dichlorophenacyl)glutathione **2b**, 276nm, -1.48 OD units; *S*-(4'-fluorophenacyl)glutathione **3b**, 266nm, -2.43 OD units.

The pH dependency of the reaction was determined with a series of buffers used previously for the characterization of glucose 6-phosphate dehydrogenase variants (21). For the determination of kinetic constants, the concentration of *S*-(phenacyl)glutathione was varied between 1 and 0.03 mM. The inhibition of the reaction by glutathione was studied by varying the concentration of glutathione between 0 and 10 mM and varying the concentration of *S*-(phenacyl)glutathione between 1 and 0.03 mM; the 2-mercaptoethanol concentration was kept constant at 10 mM.

Glutathione transferase activity with 1-chloro-2,4-dinitrobenzene (CDNB) and glutathione as substrates was determined by a previously described spectrophotometric method (22).

Protein determinations

Protein concentrations were measured by the method of Bradford (23) with bovine serum albumin as the standard.

Results

GSTO1-1 has SPG-R activity

We first noted that the reported subunit molecular size of rat liver SPG-R after SDS-PAGE was 30–37 kDa (19), which is similar to the apparent subunit size of human GSTO1-1 (31 kDa) determined under similar conditions (2). Further comparison revealed that the amino-acid composition of rat SPG-R was similar to the amino-acid composition of rat GSTO1 deduced from a cDNA clone (24), but differed from the composition of other GSTs (25) (Table 1). There were some minor differences in the His, Ile, Pro and Arg content but these variations may be attributed to experimental error in the determination of the rat SPG-R values. Because glutathione is a substrate for rat SPG-R and because of the physical similarities of SPG-R with GSTO1-1, we postulated that SPG-R and GSTO1-1 may be identical. To test this hypothesis, we tested recombinant human GSTO1-1 for SPG-R activity with Assay I (see Materials and Methods). In an initial experiment, significant activity was detected when 2,2',4'-trichloroacetophenone **2a** and glutathione were used as substrates (Table 2). Further studies with additional α -haloketones showed that whereas 2-chloroacetophenone **1a** and 2-chloro-4'-fluoroacetophenone **3a** were also substrates, no activity was observed with 3-chloropropiophenone **4a** as the substrate, indicating that the reaction is selective for α -haloketones (Table 2). The previously described rat SPG-R exhibited similar substrate selectivity (19). Several previously identified polymorphic variants of hGSTO1-1 were tested, including variants with a deletion of Glu₁₅₅. All the naturally occurring isoforms of hGSTO1-1 showed similar specific activities in Assay I (Table 3). In contrast, a C32A mutant, which lacked the active-site Cys₃₂, was inactive.

Development of Assay II

The previous study of rat SPG-R noted that if *S*-(phenacyl)glutathiones were used as the primary substrate, other thiols, such as 2-mercaptoethanol or cysteine, could replace glutathione. To determine if this was also true for the reaction catalyzed by GSTO1-1, we

developed a new direct spectrophotometric method that does not depend on the formation of glutathione disulfide; a reaction was detected in the absence of glutathione. We used the reagents listed in Assay II (see Materials and Methods) and recorded the absorbance spectra from 240 to 400 nm at timed intervals. This experiment revealed a time-dependent decrease in absorbance at 271 nm (Figure 3). In the absence of hGSTO1-1, little change in absorbance at 271 nm over the same time period was observed, indicating that the nonenzymatic reaction is negligible. The absorbance minimum and absorptivity were determined for *S*-(phenacyl) glutathione **1b**, *S*-(2,4-dichlorophenacyl)glutathione **2b**, and *S*-(4-fluorophenacyl)glutathione **3b** (see methods).

Glutathione is not essential for the SPG-R reaction

When 2-mercaptoethanol was substituted for glutathione in Assay II, hGSTO1-1 showed SPG-R activity; indeed, the specific activity was significantly increased compared with glutathione (Table 4). The increase in specific activity was highest (15-fold) with *S*-(2,4-dichlorophenacyl) glutathione **2b** as the primary substrate. Further kinetic analysis of the two reactions revealed a 5-fold increase in the k_{cat} when 2-mercaptoethanol was used in the place of glutathione in reactions with *S*-(phenacyl)glutathione **1b** as a substrate (Table 5). The kinetic analysis of the reaction was complicated by inhibition of activity when the *S*-(phenacyl)glutathione **1b** concentration was 1 mM or higher. The K_m for glutathione in Assay II was high (>30 mM) and could not be accurately determined because of difficulty in saturating the enzyme. These results are similar to the previous observation that thiols, such as 2-mercaptoethanol and cysteine, can replace glutathione in the reduction of *S*-(phenacyl)glutathiones by rat liver SPG-R (19). This finding supports the view that the previously described rat SPG-R is probably an omega-class GST.

Cys₃₂ is required for the SPG-R activity of GSTO1-1

Previous crystallographic and mutagenic studies demonstrated that Cys₃₂ is located in the active site of hGSTO1-1 and is essential for its thioltransferase and monomethylarsonate reductase activities. We found that a C32A mutant of GSTO1-1 lacked SPG-R activity, thereby confirming that Cys₃₂ is the primary active-site residue (Table 3).

Proposed reaction mechanism

The proposed reaction mechanism shown in (Figure 4) indicates that the enzyme binds *S*-(phenacyl)glutathiones at the glutathione binding site and that the active-site Cys₃₂ thiol attacks the thiol of the *S*-(phenacyl)glutathione substrate to give the enolate of acetophenone **5** and a mixed disulfide with the glutathione and Cys₃₂ of GSTO1-1. In the second step, a thiol, such as glutathione (*in vivo*) or 2-mercaptoethanol in Assay II, reacts with the active-site disulfide formed by reaction of a *S*-(phenacyl)glutathione with Cys₃₂ and forms a disulfide with the displaced glutathione. The enolate of acetophenone is readily protonated to give the observed acetophenone **6** (26). Previous studies demonstrated that the acetophenone formed in the reaction is labeled with a proton from the medium and that glutathione disulfide is formed when glutathione is incubated with 2,2',4'-trichloroacetophenone **2a** (18). Furthermore, there is chemical precedent for the reduction of α -carbonyl sulfides, *e.g.*, *S*-(phenacyl)glutathiones, by thiolates (27)

The possibility that glutathione or the glutathione-2-mercaptoethanol mixed disulfide product of the reaction in Assay II acts as an inhibitor of the reaction by nonproductively competing with *S*-(phenacyl)glutathione **1b** for the glutathione binding site on GSTO1-1 was considered. Such inhibition could explain the low SPG-R activity when glutathione was used as a cosubstrate. Similarly, the increased formation of glutathione disulfide might also explain the inhibition of Assay II by high concentrations of *S*-(phenacyl)glutathione **1b**. When glutathione was added to Assay II along with 2-mercaptoethanol, glutathione was a weak mixed inhibitor

($K_i \approx 25\text{mM}$). This degree of inhibition is probably insufficient to explain the relatively low activities observed when glutathione is used as the thiol cosubstrate.

Determination of the pH optimum of the reaction

A range of buffers was used to determine the SPG-R activity of GSTO1-1 from pH 5 to pH 11.5. The data in Figure 5 show that the optimum activity was found at approximately pH 9, which is consistent with the pK_a of glutathione in solution of 8.63 and is also consistent with the proposal that the reduction of the Cys₃₂-glutathione mixed disulfide (Figure 4) by glutathione or 2-mercaptoethanol may be the rate limiting step in the reaction. It is likely that the decreasing activity at higher pH results from conformational changes and increasing instability of the enzyme.

SPG-R activity of other cytosolic GSTs

It was of interest to determine whether other cytosolic GSTs exhibited SPG-R activity. GSTO2-2 has 64 % amino acid identity to GSTO1-1 including an active-site Cys residue. No SPG-R activity could be detected with recombinant hGSTO2-2 (data not shown). Furthermore, chloride intracellular channel protein 2 (CLIC-2), which is related to the omega-class GSTs and has a Cys residue in an analogous active-site region (28), was studied; again, this protein lacked detectable SPG-R activity (data not shown). Other GST isoenzymes that have tyrosine or serine in their active site were predicted to lack SPG-R activity. This prediction was confirmed by the observation of the lack of SPG-R activity for hGSTA1-1, hGSTA3-3, hGSTA4-4, hGSTM1-1, hGSTM2-2, hGSTM3-3, hGSTT2-2, and hGSTZ1-1 (data not shown).

SPG-R activity in tissue extracts

The apparent selectivity of the SPG-R reaction catalyzed by GSTO1-1 indicated that *S*-(phenacyl)glutathiones may be useful as selective substrates for the measurement of GSTO1-1 activity in the presence of other GSTs. Hence, SPG-R activity was quantified with Assay II and *S*-(2',4'-dichlorophenacyl)glutathione **2b** as the substrate in soluble extracts of the T47-D breast cancer cell line. Unlike other breast cancer cell lines, T47-D cells do not express GSTO1-1 (29). SPG-R activities were compared in T47-D cells that had been transfected with pcDNA3 (control) and in T47-D cells that had been transfected with GSTO1 cDNA in the pcDNA3 expression vector. As shown in Figure 6, T47-D control cells showed no immunologically detectable GSTO1 protein and lacked detectable SPG-R activity. In contrast, the Western blot shows the presence of GSTO1-1 expression, and significant SPG-R activity was observed in cells transfected with GSTO1 cDNA. The bulk GST activity with CDNB as the substrate represents the combined activity of alpha-, mu-, and pi-class GSTs present in T47-D cells. Despite the presence of other GSTs that catalyze the reaction with CDNB, only cells that express GSTO1-1 show SPG-R activity.

Previous studies of several mouse tissues indicated that GSTO1 mRNA is relatively abundant in the liver and kidney (3). When SPG-R activity was measured in liver and kidney extracts from BALBc mice with *S*-(2',4'-dichlorophenacyl)glutathione **2b** as the substrate, activities that ranged from 15 to 30 nmol min⁻¹ mg protein⁻¹ were observed. This level of SPG-R activity is at the limit of reliable detection with crude unpurified samples. Thus, although this method appears to be selective for SPG-R activity, it is not sufficiently sensitive to determine the activity of endogenous GSTO1-1 in crude tissue extracts.

Discussion

Comparison of subunit size and amino-acid composition as well as their capacity to catalyze the SPG-R reaction strongly indicate that the enzyme previously described as SPG-R and

GSTO1-1 are the same. Specifically, the activity of human GSTO1-1 with 2,2',4'-trichloroacetophenone **2a** and glutathione as substrates (0.82 units/mg) is similar to that reported for rat SPG-R (1.0 units/mg) (19). In addition, both enzymes can use a thiol other than glutathione in reactions with S-(phenacyl)glutathiones, indicating a similar reaction mechanism. Unfortunately, purified rat enzyme and antiserum to rat SPG-R are no longer available to enable an immunological comparison.

This study has revealed a novel enzymatic activity for GSTO1-1 that appears to be highly selective and distinct from reactions catalyzed by other members of the cytosolic GST structural family. Even GSTO2-2, a member of the same evolutionary class and the most closely related GST that also shares a similar active-site Cys residue, was devoid of SPG-R activity. Although knowledge of this reaction expands the range of detoxification reactions that are catalyzed by GSTs, it is not immediately clear if there is an endogenous substrate that utilizes this reaction. It is also possible that thiols other than glutathione could react with α -haloketones to form other S-phenacyl compounds however it is unlikely that they would be substrates for GSTO1-1 as they would probably not bind in the glutathione binding site.

The proposed reaction mechanism (Figure 4), which involves the formation of a mixed disulfide with the active-site cysteine₃₂ residue and its regeneration by an unbound thiol in a thiol-disulfide exchange reaction, appears to be novel within the GST family. The selectivity of the SPG-R reaction catalyzed by GSTO1-1 is demonstrated by the absence of activity in GSTO1-1 deficient T47-D cells despite the presence of other GSTs that catalyze conjugation reaction with CDNB, and the failure of a range of recombinant GSTs from several different classes to catalyze the SPG-R reaction. This is the first specific reaction attributed to GSTO1-1. Previous studies have shown that GSTO1-1 catalyzes thioltransferase, dehydroascorbate reductase, and monomethylarsonate (V) reductase activities, but these reactions are also catalyzed to varying degrees by other enzymes (3). Thus the SPG-R reaction may be of use in determining GSTO1-1 activity in the presence of a complex mixture of other GSTs.

It has previously been proposed that the reaction of α -haloketones with glutathione and their subsequent conversion to acetophenones via SPG-R activity affords protection against the toxic properties of those compounds. Individual variation in GSTO1-1 activity could alter an individual's capacity to detoxify α -haloketones. Because we have previously reported several genetic variants of GSTO1 with some different biochemical properties (4,6), we tested their specific SPG-R activities. Although all of the variants studied had specific activities that were similar to the wild-type enzyme, our previous studies have shown that the relatively rare deletion of Glu₁₅₅ results in decreased stability, indicating that individuals with this allele may exhibit a relative SPG-R deficiency.

In summary, the observation of SPG-R activity is the first specific reaction attributed to GSTO1-1 and further demonstrates its catalytic diversity.

Acknowledgements

This research was supported by grant 366731 from the National Health and Medical Research Council of Australia (P.G.B.) and by grant ES03127 from the National Institute of Environmental Health Sciences (M.W.A.). The authors thank Ms Jean Cappello for technical assistance and Leif Olson and Jalil Shojaie for their assistance in recording and interpreting the NMR and mass spectra.

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Abbreviations used

SPG-R

S-(phenacyl)glutathione reductase

Figure 1. Reaction mechanism originally proposed for the glutathione-dependent, enzymatic reduction of 2,2',4'-trichloroacetophenone (TCAP) to 2,2',4'-dichloroacetophenone (DCAP). Modified from Ahktar (17) and Brundin et al. (20).

Figure 2.

Structures of precursor α - and β -haloketones, *S*-(phenacyl)glutathiones, and *S*-(2-benzoyl)ethylglutathione. **1a**, 2-chloroacetophenone, X₁ = X₂ = H; **1b**, *S*-(phenacyl) glutathione, X₁ = X₂ = H; **2a**, 2,2',4'-trichloroacetophenone, X₁ = X₂ = Cl; **2b**, *S*-(2',4'-dichlorophenacyl)glutathione, X₁ = X₂ = Cl; **3a**, 2-chloro-4'-fluoroacetophenone, X₁ = F, X₂ = H; **3b**, *S*-(4'-fluorophenacyl)glutathione, X₁ = F, X₂ = H; **4a**, 3-chloropropiophenone; **4b**, *S*-(2-benzoyl)ethylglutathione.

Figure 3. hGSTO1-1 catalyzed reduction of *S*-(phenacyl)glutathione **1b**. *S*-(Phenacyl)glutathione **1b** and 2-mercaptoethanol were incubated at 37 °C, and spectra were recorded at 1 min intervals, as described in Assay II (see Materials and Methods).

Figure 4. Proposed reaction mechanism for the hGSTO1-1-catalyzed reduction of *S*-(phenacyl) glutathiones to acetophenones. **1b**, *S*-(phenacyl)glutathione, **2b**, *S*-(2',4'-dichlorophenacyl) glutathione, **3b**, *S*-(4'-fluorophenacyl)glutathione, **5**, keto-enol forms of product acetophenones, **6**, product acetophenones.

Figure 5. Effect of pH on the SPG-R activity of hGSTO1-1. SPG-R activity was determined with *S*-(phenacyl)glutathione **1b** and 2-mercaptoethanol as substrates in Assay II. Details of the buffers used have been described (21).

Figure 6.

Expression of hGSTO1-1 in T47-D breast cancer cells. Panel A, Western blot of cell extracts probed with antiserum to human recombinant hGSTO1-1. Panel B, GST activity of cell extracts with CDNB as the substrate. Panel C, SPG-R activity of cell extracts determined with Assay II. 1, T47-D cells transfected with pcDNA3. 2, T47-D cells transfected with hGSTO1cDNA cloned in pcDNA3. Data are shown as means $\pm$ SD.

Table 1
Comparison of the Amino-acid Composition of Rat Hepatic *S*-Phenacylglutathione Reductase, Rat GSTO1-1, and Human GSTZ1^a

Residue	S-PGR	rGSTO1
Ala	17.8	16
Cys	4	5
Asp	21.3	11 (19)
Glu	29.5	22 (29)
Phe	16.8	14
Gly	15	12
His	8.9	5
Ile	8.2	13
Lys	22.6	21
Leu	20.5	24
Met	5.6	8
Asn	-	8
Pro	13	17
Gln	-	7
Arg	13.1	10
Ser	16	16
Thr	10.6	10
Val	7.3	9
Trp	-	4
Tyr	9.9	9

^aThe S-PGR values are from Kitada et al. (19), and the rGSTO1 values were calculated from the SwissProt sequence Q9z339. The values in parentheses show Asp + Asn or Glu + Gln.

Table 2
Substrate Selectivity of the *S*-(Phenacyl)glutathione Reductase Activity of GSTO1-1^a

Substrate	Activity ($\mu\text{mol min}^{-1} \text{mg protein}^{-1}$)
2-Chloroacetophenone 1a	2.96 $\pm$ 0.13
2,2',4'-Trichloroacetophenone 2a	0.82 $\pm$ 0.03
2-Chloro-4'-fluoroacetophenone 3a	2.67 $\pm$ 0.13
3-Chloropropiophenone 4a	<0.01

^aData are shown as means $\pm$ SD of at least 3 observations. Rates were determined with Assay I.

Table 3
S-(2',4'-Dichlorophenacyl)glutathione Reductase Activity of Human GSTO1-1 Variants^a

Enzyme	Specific activity ($\mu\text{mol GSSG formed min}^{-1}\text{mg protein}^{-1}$)
hGSTO1-1	1.3 $\pm$ 0.1
hGSTO1-1 Δ E155, E208	1.3 $\pm$ 0.05
hGSTO1-1 Δ E155, K208	1.1 $\pm$ 0.03
hGSTO1-1 K208	1.1 $\pm$ 0.07
hGSTO1-1 D140	1.1 $\pm$ 0.05
hGSTO1-1 A32	<0.01
rat SPG-R	1.0 $\pm$ 0.03

^aThe substrate was 2,2',4'-trichloroacetophenone **2a**; reaction rates were determined with Assay I (Materials and Methods). The data are expressed as means $\pm$ SD. Reported rat hepatic SPG-R activity (19) is shown for comparison.

Table 4**Substrate Selectivity of the *S*-Phenacylglutathione Reductase Activity of hGSTO1-1^a**

Substrate	Glutathione ($\mu\text{mol mg protein}^{-1} \text{ min}^{-1}$)	2-Mercaptoethanol ($\mu\text{mol mg protein}^{-1} \text{ min}^{-1}$)
<i>S</i> -(Phenacyl)glutathione 1b	3.9 $\pm$ 0.33	11.1 $\pm$ 0.1
<i>S</i> -(2',4'-Dichlorophenacyl)glutathione 2b	1.2 $\pm$ 0.01	15.3 $\pm$ 0.26
<i>S</i> -(4-Fluorophenacyl)glutathione 3b	3.6 $\pm$ 0.36	7.4 $\pm$ 0.15
<i>S</i> -(Propiophenacyl)glutathione 4b	<0.01	<0.01

^aReaction rates were determined with Assay II (Materials and Methods). Data are shown as means $\pm$ SD of at least 3 observations.

Kinetic Parameters for GSTO1-1 with S-(Phenacyl)glutathione 1b and Glutathione and 2-Mercaptoethanol as Thiol Substrates^a

	Glutathione			2-Mercaptoethanol		
	k_{cat} (S ⁻¹)	K_m^{SPG} (mM)	k_{cat}/K_m (M ⁻¹ S ⁻¹)	k_{cat} (S ⁻¹)	K_m^{SPG} (mM)	k_{cat}/K_m (M ⁻¹ S ⁻¹)
GSTO1-1	0.8 ± 0.4	0.062 ± 0.01	1.3 × 10 ⁴	5.8 ± 0.47	0.33 ± 0.05	1.76 × 10 ⁴

^a Reaction rates were determined with Assay II (Materials and Methods). Data are shown as means ± SD of at least 3 observations.