THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 285, NO. 8, pp. 5639–5645, February 19, 2010

Minor Modifications of the C-terminal Helix Reschedule the Favored Chemical Reactions Catalyzed by Theta Class Glutathione Transferase T1-1*[S]

Received for publication, October 9, 2009, and in revised form, December 8, 2009 Published, JBC Papers in Press, December 18, 2009, DOI 10.1074/jbc.M109.074757

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Adaptive responses to novel toxic challenges provide selective advantages to organisms in evolution. Glutathione transferases (GSTs) play a pivotal role in the cellular defense because they are main contributors to the inactivation of genotoxic compounds of exogenous as well as of endogenous origins. GSTs are promiscuous enzymes catalyzing a variety of chemical reactions with numerous alternative substrates. Despite broad substrate acceptance, individual GSTs display pronounced selectivities such that only a limited number of substrates are transformed with high catalytic efficiency. The present study shows that minor structural changes in the C-terminal helix of mouse GST T1-1 induce major changes in the substrate-activity profile of the enzyme to favor novel chemical reactions and to suppress other reactions catalyzed by the parental enzyme.

The molecular evolution of enzyme functions encompasses redesign of existing structures as one of the cornerstones (1). Structural studies and evolutionary analyses give ample examples of structural scaffolds that have been adopted for diverse functions (2). Many enzymes have evolved to acquire high selectivity for naturally occurring substrates, but enzymes involved in the cellular detoxication system generally have a broad substrate acceptance, or promiscuity (3). Proteins have high structural flexibility, and in some enzymes the active site can undergo major conformational changes and thereby accommodate substrates of widely different shapes, as exemplified by cytochrome P450 3A4 (4). Glutathione transferases (GSTs)[2] form a superfamily of enzymes encompassing seven classes of canonical soluble enzymes (5). Some of the GSTs have evolved for intermediary metabolism, such as the biosynthesis of steroid hormones (6) and prostaglandins (7), or the degradation of aromatic acids (8). However, the majority of the soluble GSTs appear to have their primary function in the inactivation of toxic compounds from exogenous as well as endogenous sources.

Surprisingly, structurally highly similar GST homologs in the same biological organism or from diverse species differ in their substrate selectivity profiles. A primary determinant for divergent substrate acceptance among GSTs appears to be the H-site, *i.e.* the binding site for the electrophilic substrate (9, 10). On the other hand, GST A4-4 in rats and mice are structurally quite different from the human counterpart, even though their catalytic efficiencies are similarly evolved for high activity with the cytotoxic 4-hydroxynonenal generated by lipid peroxidation (11). These findings suggest that the GST structure is functionally malleable such that catalytic properties can readily be altered in a relatively short evolutionary time span. In the present investigation we demonstrate how simple point mutations and a deletion in the C-terminal helix of Theta class GST (GST T1-1) can drastically alter the substrate specificity profile with consequent increases in activity with certain substrates and decreases with other substrates. This shift in chemistry of the favored reaction suggests that minor changes in the C-terminal helix of GST T1-1 could play an important role in adaptive responses to novel chemical challenges in the evolution of an organism as well as in protection of cells and tissues against emerging toxic insults.

EXPERIMENTAL PROCEDURES

Site-directed Mutagenesis—Mouse GST T1-1 was previously cloned from a cDNA library (12). The coding sequence was extended in the 5′ end to comprise codons for an N-terminal His_6 tag. The plasmid pKKD (13) was used for protein expression in *Escherichia coli*. The coding sequence in the same vector was the template mutated to produce the M232A and R234W mutants. For preparation of the double mutant M232A/R234W, the point-mutated plasmid pKKD (R234W) was the template. The oligonucleotides were purchased from Thermo Scientific (sequences read from left to right in 5′ to 3′ direction), and the site of mutagenesis is underlined: M232A forward (24-mer), AAG CTG GCT CCC AGA GTG CTG GCA and M232A reverse (23-mer), CTG CTT TAT GAT GAG GTC AGC AG. The variant R234W forward (24-mer) is ATG CCC TGG GTG CTG GCA ATG ATC, and R234W reverse (26-mer) is CAG CTT CTG CTT TAT GAT GAG GTC AG. M232A/R234W forward (24-mer) is AAG CTG GCT CCC TGG GTG CTG GCA, and the reverse primer sequence is CTG CTT TAT GAT GAG GTC AGC AG. The deletion mutant ΔR234W was

* This work was supported by the Swedish Research Council and the Swedish Cancer Society.

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Tables S1 and S2.

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[2] The abbreviations used are: GST, glutathione transferase; EPNP, 1,2-epoxy-3-(4-nitrophenoxy)-propane; NPB, 4-nitrophenethyl bromide; phenethyl-ITC, phenethyl isothiocyanate; benzyl-ITC, benzyl isothiocyanate; allyl-ITC, allyl isothiocyanate; propyl-ITC, propyl isothiocyanate; H_2O_2, hydrogen peroxide; *tert*-BuOOH, tertiary butyl hydroperoxide; CuOOH, cumene hydroperoxide.

obtained as a side product in the preparation of the R234W mutant. The mutagenic reaction was run in a total volume of 100 μl containing 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 5 units/ml of *Pfu* Turbo polymerase and buffer, as recommended by the manufacturer (Stratagene, La Jolla, CA). The amplified cDNA product was digested with EcoRI and HindIII, run on a 1% (w/v) agarose gel, and purified using QIAquick (Qiagen, Hilden, Germany). The digested PCR product was finally ligated to the EcoRI and HindIII cloning sites of pKKD.

Expression and Purification of GST T1-1 Variants—To express and purify His-tagged wild type mouse GST T1-1 as well as its variants (M232A, M232A/R234W, R234W, and ΔR234W), cultures were grown at 37 °C in 2TY medium (1.6% (w/v) tryptone, 0.1% (w/v) yeast extract, 0.5% (w/v) NaCl) supplemented with 100 mg/ml ampicillin until the culture reached an A_{600} of 0.2. Isopropyl-β-D-thiogalactopyrasoside was added to a final concentration of 0.1 mM, and the culture was allowed to grow for a minimum of 18 h. The bacteria were collected by centrifugation and resuspended in buffer A (20 mM sodium phosphate, 500 mM NaCl, pH 7.4, 85 mM imidazole, and 10 mM 2-mercaptoethanol) containing 0.2 mg/ml lysozyme. After standing on ice for 1 h, the bacteria were ultrasonicated and centrifuged at 30,000× *g* for 30 min at 4 °C. The supernatant fraction containing the recombinant enzyme was loaded on a Ni-IMAC affinity column. Unbound protein was washed out with buffer A. The GST was eluted with buffer A supplemented with 500 mM imidazole. The eluted enzyme was dialyzed using 10 mM Tris-HCl, pH 7.8, 1.0 mM 2-mercaptoethanol, and 20% (v/v) glycerol. The homogeneity of the pooled material was analyzed with SDS-PAGE, using 12.5% (w/v) polyacrylamide gel (14). Protein bands were visualized by staining with Coomassie Brilliant Blue R-250. The purified enzyme was stored at −80 °C.

Assay of Enzymatic Activities of Variants of GST T1-1—The activities of purified wild type mouse GST T1-1 and four variants (M232A, M232A/R234W, R234W, and ΔR234W) were measured at 30 °C as published previously using the signature substrates 1,2-epoxy-3-(4-nitrophenoxy)-propane (EPNP), 4-nitrophenethyl bromide (NPB) (15), and iodomethane (16). Alternative substrates included the phenethyl isothiocyanate (phenethyl-ITC), benzyl isothiocyanate (benzyl-ITC), allyl isothiocyanate (allyl-ITC), propyl isothiocyanate (propyl-ITC) (17), hydrogen peroxide (H_2O_2), tertiary-butyl hydroperoxide (*tert*-BuOOH), and cumene hydroperoxide (CuOOH) (18) as well as different monoiodoalkanes and diiodoalkanes (16). The assay conditions are summarized in supplemental Table S1.

Molecular Modeling—Structural models of mouse GST T1-1 and the mutated variants were obtained using MODBASE (19). The structures of wild type human GST T1-1 as well as its W234R variant in complex with *S*-hexylglutathione (Protein Data Bank codes 2c3n and 2c3q, respectively) were used as guides. Structural models were visualized using the Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (20).

RESULTS

Mutations in the C-terminal Helix—GST T1-1 in the mouse is 82% sequence identical with human GST T1-1, and the

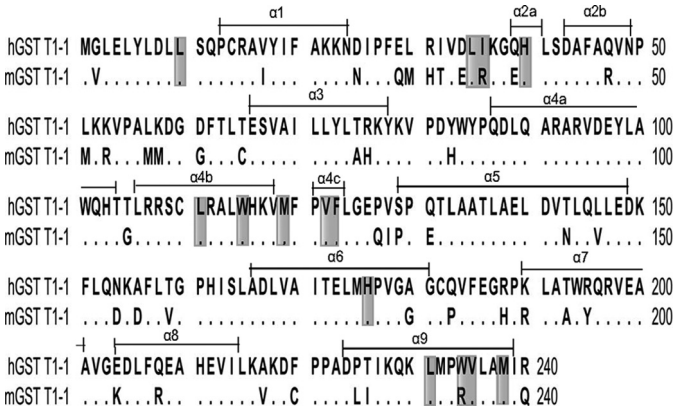


FIGURE 1. **Comparison of wild type mouse GST T1-1 (*mGST T1-1*) and human GST T1-1 (*hGST T1-1*) sequences.** The different α-helix regions indicated are based on the crystal structure of hGST T1-1 (Protein Data Bank code 2c3n) (24). H-site residues are marked with *gray boxes*. The G-site residues (not indicated) are conserved between human and mouse GST T1-1.

enzymes are obviously homologous (Fig. 1). The amino acid residues forming the binding sites for glutathione (G-site) and the electrophilic second substrate (H-site) are essentially conserved. Two H-site residues differ in positions 36 and 234, but residue 36 is peripheral in the site, ~10 Å distant from the sulfur of glutathione. However, an important difference occurs in one of the residues located in the C-terminal helix. In position 234, the human enzyme has Trp, whereas the mouse enzyme has Arg. We have previously shown that mutation of Trp into Arg in the human enzyme induces significant elevation of catalytic activity with a number of substrates (16). To test if the reverse mutation, Arg to Trp, in a homologous enzyme is sufficient to suppress catalytic activities, this substitution was introduced in mouse GST T1-1. In the course of mutagenesis, a spurious deletion of Met^{232} was obtained, and the properties of the expressed enzyme indicated that also residue 232 strongly influences catalytic activity, without being in the first coordination sphere of any of the substrates. Met^{232} was therefore mutated into Ala in both the wild type mouse GST T1-1 and the mutant R234W. Like wild type GST T1-1, the mutants were stable proteins that could readily be expressed in *E. coli*.

Substrate Activity Profiles of GST T1-1 Variants—GSTs catalyze a wide variety of reactions with electrophilic substrates including diverse chemical mechanisms. Fig. 2 shows the compounds used in the present investigation, representing substitution, reduction, as well as different addition reactions. Fig. 3 and supplemental Table S2 document the measured activities and demonstrate that the wild type enzyme GST T1-1 and the four mutants M232A, M232A/R234W, R234W, and ΔR234W differ widely in their substrate-activity profiles.

Replacement of Arg^{234} by Trp Universally Attenuates Catalytic Activity—The R234W mutation significantly decreased the activity compared with wild type GST T1-1 with all substrates tested. The epoxide EPNP, the alkylhalide iodomethane, and NPB have often been regarded as signature substrates of GST T1-1 (16, 21). Indeed, the R234W activities with these substrates were still the highest in absolute numbers but just <3% of the wild type values. On the other hand, some of the naturally occurring GST substrates, such as phenethyl-ITC,

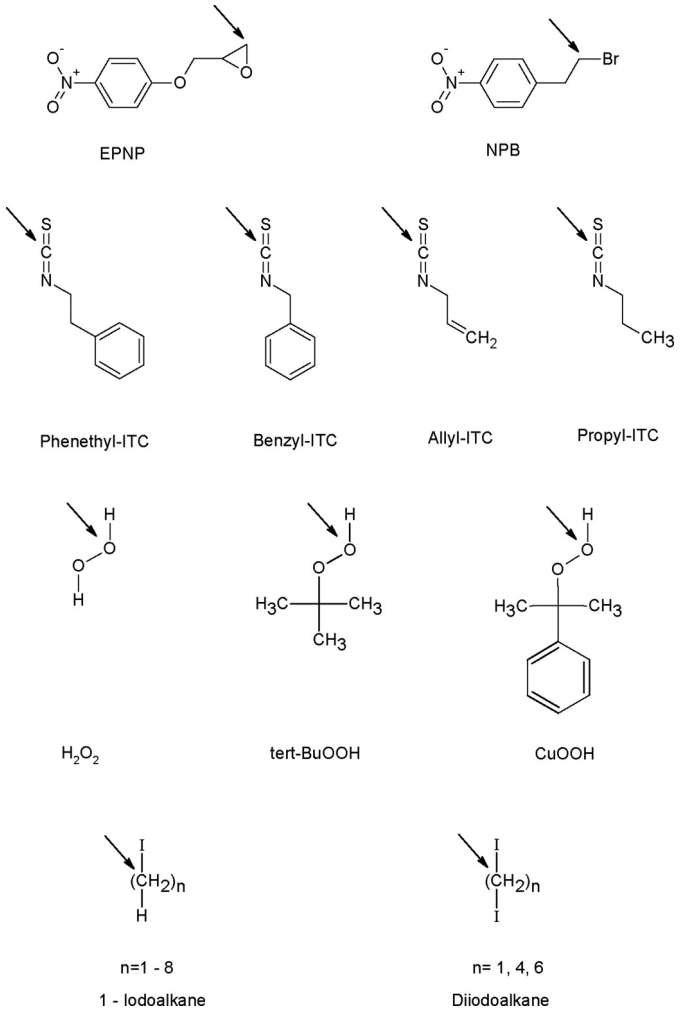


FIGURE 2. **Structures of alternative electrophilic substrates used in activity measurements.** *Arrows* indicate the site of attack on the substrates: EPNP, NPB, phenethyl-ITC, allyl-ITC, propyl-ITC, benzyl-ITC, H_2O_2, *tert*-BuOOH, and CuOOH. See supplemental Table S1 for assay conditions.

benzyl-ITC, and H_2O_2, retained ~20% of the activities of the wild type enzyme.

Deletion of Residue 232 in Mutant R234W Causes Further Attenuation of Signature Activities but Elevated Values with Alternative Substrates—Deletion of Met^{232} from mutant R234W, to give the double mutant ΔR234W, led to further 2.5–20-fold decreases in the activities with the signature substrates. By contrast, the deletion conferred higher activities with substrates other than those displayed by R234W. CuOOH was the favored substrate of ΔR234W, which showed 47-fold higher activity than R234W and even 16-fold higher than wild type GST T1-1 in the reduction of this hydroperoxide.

Replacement of Met^{232} by Ala Enhances Activity with Several Substrates—The activities of M232A with the signature substrates were moderately lower by 3–7-fold compared with the wild type values. Strikingly, the CuOOH activity was 30-fold elevated by the M232A substitution. Mutant M232A displayed the highest activity with CuOOH of all enzyme variants tested. Another substrate transformed with similar high activity by M232A was allyl-ITC, showing 7-fold higher activity than wild type GST T1-1.

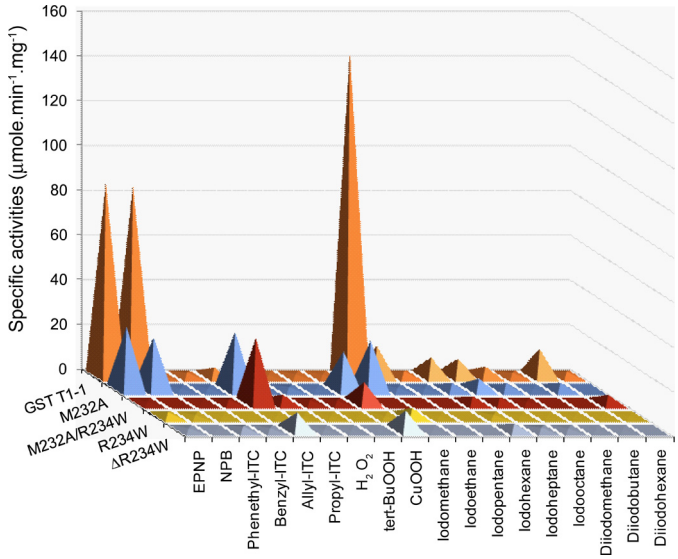
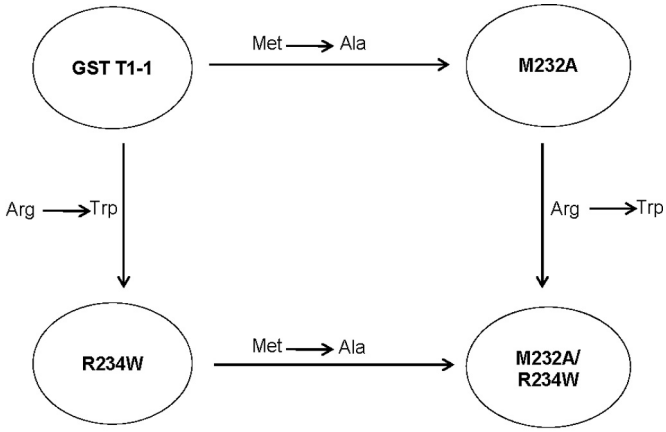


FIGURE 3. **Pyramid plot of catalytic activity profiles of wild type mouse GST T1-1 and variants M232A, M232A/R234W, R234W, and ΔR234W with 18 alterative electrophilic substrates.** The substrates used were EPNP, NPB, phenethyl-ITC, allyl-ITC, propyl-ITC, benzyl-ITC, H_2O_2, *tert*-BuOOH, CuOOH, and different (mono- and di-) iodoalkanes. The specific activity values are given in supplemental Table S2.

The Double Mutant M232A/R234W Has the Lowest Activity with the Signature Substrates but Has the Highest with Allyl- and Propyl-ITC—The double mutation caused a loss of the activity by several orders of magnitude with the signature substrates. NPB did not reveal any measurable enzymatic reaction, and iodomethane showed a 3,000-fold lower activity than with the wild type enzyme. In absolute numbers, mutant M232A/R234W displayed the highest activity with allyl-ITC, similar to the activity of mutant M232A and 8-fold higher than the wild type value. In comparison with all other GST T1-1 variants, M232A/R234W was clearly the most effective in the conjugation of propyl-ITC, 16-fold more active than wild type GST T1-1. Thus, the double mutation causes a switch in the relative activities with iodomethane and propyl-ITC by 48,000-fold compared the wild type ratio.

Double Mutant Cycle Shows that the Effect of Mutations in Positions 232 and 234 Are Generally Not Additive—As first demonstrated by Fersht and co-workers (22), mutations of two amino acids in a protein, separately as well as combined, can identify coupling between the different positions. By measurements of proper thermodynamic parameters, quantitative relationships regarding structure and function can be determined (23). Fig. 4 displays a double mutant cycle of the two substitutions in GST T1-1 in the investigated positions, excepting the deletion, as well as the ratios of mutant activities to the corresponding wild type activities for alternative substrates. The numbers in Fig. 4 are based on specific activities and cannot be used for calculation of accurate energetic relationships. Nevertheless, the data show clearly that the effects of the mutations are generally not additive, with the possible exceptions of the activities with EPNP and NPB. In other words, the influence of a mutation in position 232 on the effect of a mutation in position 234 depends on the substrate assayed. With some substrates a second mutation reinforces and with other substrates it weakens the effect of the first mutation.



Substrate	Activity ratio to GST T1-1			
	M232A	M232A/R234W	R234W	ΔR234W
EPNP	0.33	0.006	0.025	0.01
NPB	0.28	0	0.008	0.001
Phenethyl-ITC	0.56	0.25	0.21	2.4
Benzyl-ITC	0.28	0.96	0.23	0.62
Allyl-ITC	7.0	7.9	0.05	2.2
Propyl-ITC	2.1	16	0.22	1.31
H_2O_2	2.9	3.7	0.16	0.59
tert-BuOOH	0.26	1.5	0.09	0.1
CuOOH	30	17	0.34	16

FIGURE 4. **Double mutant cycle of two residues in the C-terminal helix influencing the activity profile in GST T1-1.** The *upper part* shows a scheme of the two substitutions in GST T1-1 in the Met^{232} and Arg^{234} positions. The *lower part* shows the ratios of mutant activities to the corresponding wild type activities for alternative substrates.

Minor Structural Modifications of the C-terminal Helix Cause the Enzyme to Favor Reactions of Different Chemistries—The C terminus of GST T1-1 forms a helix that serves as a lid over the active site and brings residues in direct contact with the electrophilic substrate (24). It is assumed that the lid has to open to release the product formed in the catalyzed reaction. The structure of the wild type mouse GST T1-1 as well as the variants M232A, M232A/R234W, R234W, and ΔR234W were modeled based on the available crystal structures of human GST T1-1 and its W234R mutant. The determined structure of human GST T1-1 and the modeled structure of mouse GST T1-1 are essentially superimposable (root mean square deviation = 0.33 Å), except for those residues that differ between the two sequences, *e.g.* Trp^{234} in the active site of human GST T1-1 and Arg^{234} in the mouse enzyme. Fig. 5*A* shows the C-terminal helix of the human W234R mutant liganded with *S*-hexylglutathione (Protein Data Bank code 2c3q) and the corresponding structure of the modeled wild type mouse GST T1-1. The overlap of the two structures is equally close in the remainder of the polypeptide chains. In the human W234R mutant, Arg^{234} displays an ionic bond with the carboxyl group of glutathione (24), and the wild type mouse GST T1-1 presumably forms a similar ionic interaction (Fig. 5*A*).

Residue 232 significantly affects the catalytic activity of GST T1-1 toward different substrates as shown in Fig. 3 and supplemental Table S2, but this residue is located on the outside of the C-terminal helix, and its side chain is pointed away from the active site. The model of the mutant M232A (root mean square deviation = 0.6 Å) shows residue 232 in the

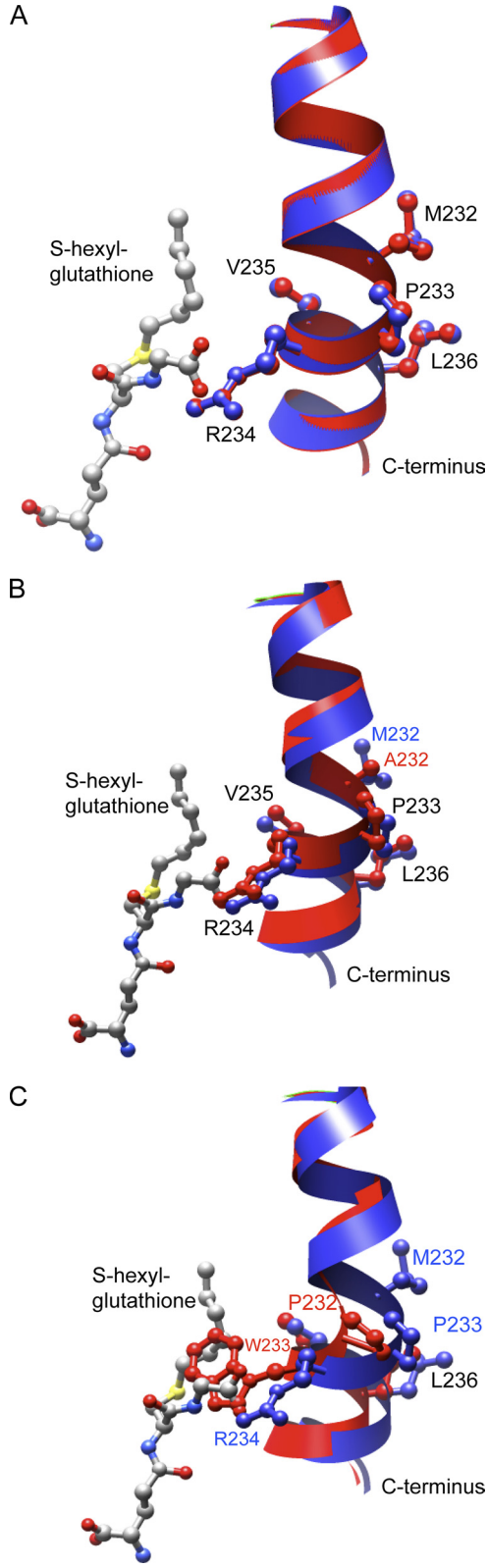


FIGURE 5. **Comparison of the C-terminal helix in wild type mouse GST T1-1 and the M232A and ΔR234W variants.** Modeled structures of mouse GST T1-1 variants (*red*) are superpositioned with the W234R mutant of human GST T1-1 (*blue*) in complex with *S*-hexylglutathione (Protein Data Bank code 2c3q). *A*, wild type mouse GST T1-1. *B*, mutant M232A. *C*, mutant ΔR234W. The amino acids Met^{232}, Pro^{233}, Arg^{234}, Val^{235}, and Leu^{236} are shown in *ball and stick* representation. In mutant ΔR234W, the numbering of Pro and Trp (*red*) has changed because of the deletion of Met^{232}. The figure was produced using the University of California, San Francisco, Chimera program.

same location and Arg^{234} placed in the position found in wild type GST T1-1 (Fig. 5*B*).

The models of M232A/R234W and R234W based on wild type human GST T1-1 were also very similar to the human structure (root mean square deviation = 0.59 Å and 0.51 Å, respectively). Thus, the differences in catalytic activity cannot be ascribed to major alterations of the polypeptide chain.

In contrast to the other structures, the model of the ΔR234W mutant (root mean square deviation = 0.87 Å) shows a small but significant alteration of the C-terminal helix. Removal of residue 232 from mutant R234W accentuates a bend of the helix following Pro^{233} in the parental structure and shifts the position of Trp^{234} in the active site (Fig. 5*C*).

DISCUSSION

Adaptation to novel habitats, altered environmental conditions, and new dietary factors may require enhanced activities of enzyme systems that provide protection against chemical challenges. An investigation of *Drosophila* populations from different habitats has demonstrated that their respective complements of GSTs differ significantly (25). *Drosophila* larvae feeding on different cacti in California and Mexico are exposed to different chemicals in the diverse plants that presumably have governed evolution of GSTs with different substrate selectivity profiles. Although most GSTs have broad substrate acceptance or catalytic promiscuity, it is clear that some GSTs have become specialized in evolution to catalyze a limited number of reactions with high catalytic efficiency. GST A4-4, for example, has acquired particularly high activity with 4-hydroxynonenal and other toxic products of lipid peroxidation (26). Another example is human GST M2-2, which is highly efficient in the inactivation of neurotoxic orthoquinones derived from dopamine and other naturally occurring catecholamines (27).

The catalytic properties of GSTs are strikingly malleable, and several examples demonstrate that simple point mutations can induce catalytic activities far above those present in the parental GST (28–30). We have previously demonstrated that human GST T1-1 has a Trp residue in position 234 that blocks the entry to the active site and suppresses activity with a variety of substrates. Replacement of Trp by Arg in this position renders the enzyme highly active with a number of substrates (16). Saturation mutagenesis of residue 234 in the same human enzyme has demonstrated that all alternative amino acids afford enhanced catalytic activity with a fluorogenic substrate (31). In the present study we show that all of the measured activities of mouse GST T1-1 are suppressed by the reverse mutation R234W in position 234. The functional changes can be rationalized by the location of residue 234 in the binding site of the electrophilic substrate, the H-site. However, residue 232, which points away from the H-site, also influences the catalytic efficiency and the substrate selectivity. Thus, novel catalytic abilities can be gained by mutations in these positions in the C-terminal helix. The explanation of the emergence of diverse activities requires detailed experimental studies of the different structures and their dynamic properties. The point mutations investigated by us can be expected to cause alterations of the H-site topology, but the changes need confirmation by direct structural studies. In closely related mammalian GST T1-1 enzymes, residue 232 is Met, Leu, or Arg, and residue 234 is Arg, Trp, Lys, or Ser, in alternative combinations. It would be of interest to investigate the functional consequences of all of these naturally occurring variations.

So far, none of the recorded allelic variants of human GST T1-1 shows sequence variations in the C-terminal region, and gain of function has not been observed (www.ncbi.nlm.nih.gov/projects/SNP). Nevertheless, the *GSTT1* chromosome locus 22q11.2 is in a region that is frequently subject to segmental duplications and rearrangements (32), and the gene could therefore have an increased tendency to undergo somatic mutations that could influence carcinogenesis and enhance drug resistance (33).

In the present study of GST T1-1, the enhanced activities with two classes of compounds may have particular biological significance: hydroperoxides and organic isothiocyanates. Hydrogen peroxide is an important byproduct of the respiratory chain in mitochondria and increases in concentration under conditions of oxidative stress and toxicity. Organic hydroperoxides are downstream products of reactive oxygen species. CuOOH and *tert*-BuOOH used in our investigation are model substrates for the far more unstable lipid hydroperoxides formed in cells. Mutant M232A displays 30-fold elevated activity with CuOOH, even though the activity with the structurally related *tert*-BuOOH is almost 4-fold decreased. By contrast, the double mutant M232A/R234W shows 1.5-fold higher activity than the wild type enzyme with *tert*-BuOOH, whereas mutant R234W has the lowest activity of all enzyme variants (Fig. 3). Deletion mutant ΔR234W displays 47-fold enhanced activity with CuOOH and 3.8-fold enhanced activity with H_2O_2 but no significant change with *tert*-BuOOH compared with R234W. Evidently, subtle interactions between substrate and the active site have major effects on substrate selectivity also in a promiscuous enzyme.

The second group of biologically relevant substrates comprises the organic isothiocyanates. They are conjugated with glutathione to form dithiocarbamates, which are eventually excreted in the form of mercapturic acids. Organic isothiocyanates are abundant in plants where they are bound as glucosinolates from which they are released when the plant tissue is injured. GST activity with organic isothiocyanates is occurring in organisms ranging from cyanobacteria (34) to man (17), indicating its biological importance. All mouse GST T1-1 mutants except R234W displayed enhanced activity with the small-side-chain isothiocyanates (allyl- and propyl-ITC). Only the deletion mutant ΔR234W had elevated activity toward an isothiocyanates with an aromatic substituent (*i.e.* phenethyl-ITC).

It can be speculated that ancestral GSTs had high activities with these biologically important classes of compounds and that these activities were subsequently sacrificed for new activities with emerging alternative substrates. The suggested primordial activities can now be readily recovered by back mutations. An alternative view is that the protein scaffold has a structure intrinsically conducive to acquire these activities.

Substrate discrimination may be more important than catalytic efficiency because the catalytic capacity can be regulated by changes of the enzyme concentration, whereas deleterious

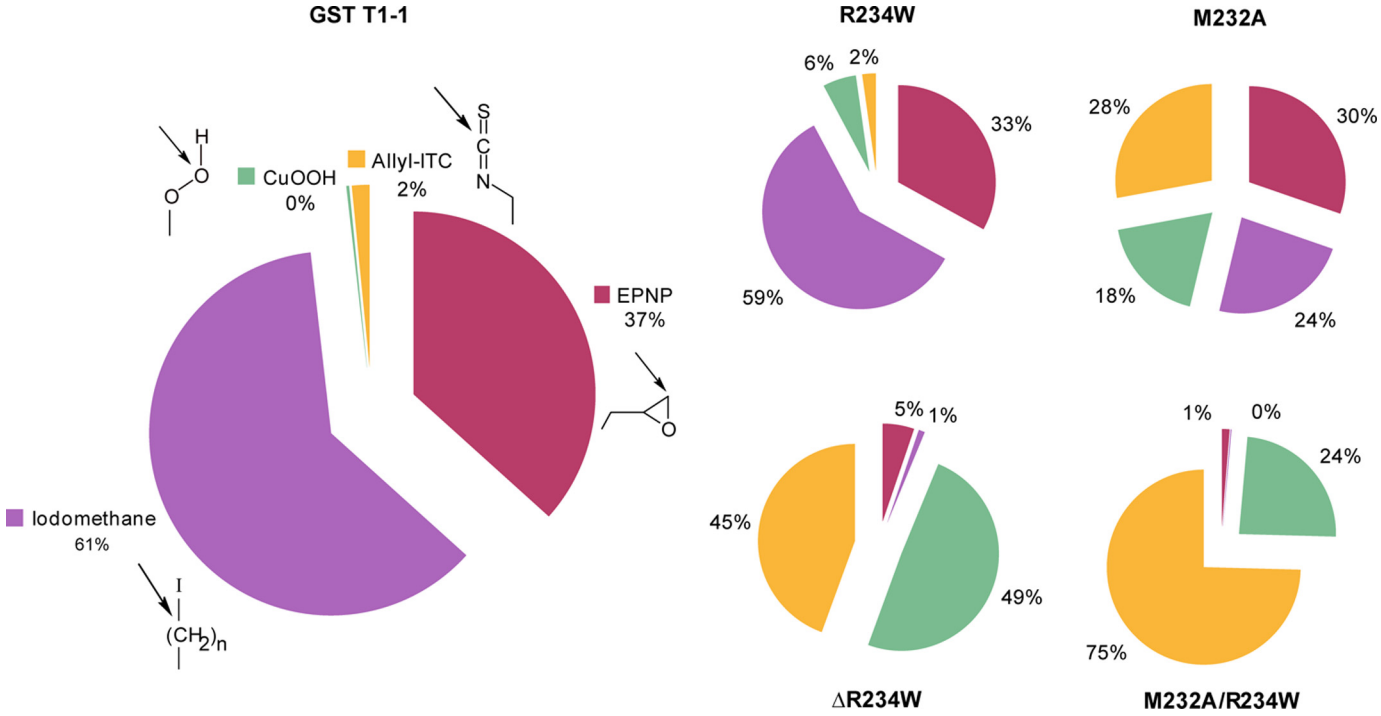


FIGURE 6. **Rescheduling of favored chemical reactions by point mutations in GST T1-1.** Pie charts show the relative activities of wild type GST T1-1 and the four variants R234W, M232A, ΔR234W, and M232A/R232W with the alternative substrates EPNP, iodomethane, CuOOH, and allyl-ITC, representing four diverse chemical reactions.

side reactions with alternative substrates can be detrimental to the function of a biochemical system (35). Fig. 6 illustrates how the investigated mutations of the mouse GST T1-1 affect the substrate discrimination among four substrates undergoing dissimilar chemical reactions with release of different numbers of product molecules. The molecular transformations involve: thiolysis of the epoxide EPNP (one *S*-1-hydroxyalkylglutathione product), alkyl substitution of iodomethane (two products, *S*-methylglutathione and iodide), addition to the isothiocyanate allyl-ITC (one *S*-dithiocarbamoyl-glutathione product), and reduction of the hydroperoxide CuOOH (three products, the alcohol cumenol, water, and glutathione disulfide).

The wild type GST T1-1 has similar activities with EPNP and iodomethane but minor activity with the other substrates. The substrate acceptance of mutant R234W is similar to that of the wild type enzyme, even though the absolute values of the activities are lower. In striking contrast to the wild type and R234W enzymes, mutant M232A has relaxed selectivity such that all four substrates have comparable activities (Fig. 6). Deletion of residue 232 reverses the wild type substrate preferences as shown by the ΔR234W mutant, which displays the highest activities with CuOOH and allyl-ITC. The highest selectivity *vis à vis* the four substrates is demonstrated by the double mutant M232A/R234W, which is dominated by the activity with allyl-ITC.

Even though Fig. 6 illustrates the switch between substrates undergoing different chemical reactions, it should be noted that the functional group as such has subordinate influence on the catalytic activity displayed by a given enzyme variant. For example, the activities noted with different homologous iodoalkanes showed no obvious structure-activity relationships, and the chemically most reactive iodomethane gave activities that varied by more than 3 orders of magnitude among the mutants (Fig. 3 and supplemental Table S2). Wild type GST T1-1 displayed the highest activity of the enzymes with almost all iodoalkanes, but mutant M232A/R234W was the most active variant with 1,6-diiodohexane.

In summary, we report remarkable alterations of the substrate specificity profile of GST T1-1 brought about by simple mutations in the C-terminal helix. The activity of the wild type enzyme was enhanced with substrates such as hydroperoxides and isothiocyanates, whereas the activity with other substrates was suppressed. The ratio of activities with two alternative substrates can change as much as 48,000-fold, and the chemistry of the favored reaction could switch among reduction of a hydroperoxide, thiocarbamoylation, opening of an epoxide, and nucleophilic substitution depending on the mutation in the enzyme and the substrate investigated. The facile shift in catalytic properties suggests that mutations in the C terminus of GST T1-1 could play an important role in adaptive responses to novel chemical challenges in the evolution of its biological host.

Acknowledgments—We thank Dr. Anna-Karin Larsson who originally cloned wild type mouse GST T1-1 and Dr. Françoise Raffalli-Mathieu and Dr. Usama Hegazy for valuable comments and discussion.

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