Human glutathione transferases (GSTs) were shown to catalyze the reductive glutathione conjugation of aminochrome (2,3-dihydroindole,5,6-dione). The class Mu enzyme GST M2-2 displayed the highest specific activity (148 μmol/min/mg), whereas GSTs M1-1, A2-2, M1-1, M3-3, and P1-1 had markedly lower activities (<1 μmol/min/mg). The product of the conjugation, with a UV spectrum exhibiting absorption peaks at 277 and 295 nm, was 4-S-glutathionyl-5,6-dihydroxyindoline as determined by NMR spectroscopy. In contrast to reduced forms of aminochrome (leucoaminochrome and o-semi-quinone), 4-S-glutathionyl-5,6-dihydroxyindoline was stable in the presence of molecular oxygen, superoxide radicals, and hydrogen peroxide. However, the strongly oxidizing complex of Mn** and pyrophosphate oxidizes 4-S-glutathionyl-5,6-dihydroxyindoline to 4-S-glutathionylaminochrome, a new quinone derivative with an absorption peak at 620 nm. GST M2-2 (and to a lower degree, GST M1-1) prevents the formation of reactive oxygen species linked to one-electron reduction of aminochrome catalyzed by NADPH-cytochrome P450 reductase. The results suggest that the reductive conjugation of aminochrome catalyzed by GSTs, in particular GST M2-2, is an important cellular antioxidant activity preventing the formation of o-semi-quinone and thereby the generation of reactive oxygen species.

**Human glutathione transferases, in particular Isoenzyme M2-2, Catalyze Detoxication of the Dopamine Metabolite Aminochrome**

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Dopamine, like other catecholamines, can be oxidized to the corresponding o-quinone (1–4). The formation of catecholamine o-quinones is followed by cyclization involving the amino group of their side chains. The reduction of aminochrome, dopa-chrome, noradrenochrome, and adrenochrome is accompanied by their subsequent reoxidation by oxygen, which gives rise to reactive chemical species (5–8). The oxidative conversion of dopamine into aminochrome (2,3-dihydroindole,5,6-dione) and its subsequent reduction and reoxidation by oxygen are believed to be the cause of neurodegenerative processes in the dopamine system (9). Degeneration of the dopaminergic neurons in the nigrostriatal system as a consequence of aminochrome activation to produce reactive chemical species has been proposed to contribute to the development of Parkinson's disease (9, 10). In addition, aminochrome-dependent neurodegeneration of the dopaminergic system in the mesolimbic system has been suggested as a contributing process in the development of schizophrenia (for reviews, see Refs. 11 and 12).

Glutathione transferases (GSTs)† catalyze the detoxication of a variety of xenobiotics including carcinogens, environmental contaminants, anticancer agents, antioxidants, and products of oxidative processes (for review, see Ref. 13). However, the ability of GSTs to catalyze conjugation of quinones is still largely unexplored. Quinones are a widespread group of xenobiotics that may give rise to cytotoxicity, mutagenesis, and carcinogenesis (14–16). The toxic effects of quinones are exploited in certain anticancer agents. On the other hand, endogenous quinones such as vitamin K and ubiquinone serve important physiological functions. In addition to their well-established roles, ubiquinone has been proposed to be an important antioxidant in biological membranes. The protective function is provided in the reduced hydroquinone state (17), generated by the two-electron reduction catalyzed by NAD(P)H:quinone oxidoreductase (18). To study the possibility that GSTs catalyze the conjugation and detoxication of quinones, aminochrome has been chosen as a representative of endogenous quinones of obvious pathophysiological significance. This work shows that human GSTs catalyze the formation of a glutathione conjugate of aminochrome, which is more resistant to redox cycling than the parent compound. Thus, glutathione conjugation appears to be an important mechanism for protection against oxidative stress.

**EXPERIMENTAL PROCEDURES**

**Chemicals**

Dopamine, NADH, NADPH, and GSH were purchased from Sigma.

**Animals**

Male Sprague-Dawley rats with body weights of ~ 100 g were used. The animals were kept on standard laboratory diet and starved for 24 h before being sacrificed. The animals used for NADPH-cytochrome P450 reductase purification were injected intraperitoneally with 8 mg of sodium phenobarbital once daily for 4 days.

**Preparation of Enzymes**

Human glutathione transferases M1-1 (allelic variant b), M2-2, M3-3, A1-1, A2-2, and P1-1 (for nomenclature, see Ref. 19) were obtained by heterologous expression in Escherichia coli and purified by affinity chromatography on S-hexylglutathione immobilized on Sephrose 4B (20). NADPH-cytochrome P450 reductase was purified from liver microsomes from phenobarbital-treated rats according to Yasukochi and Masters (21). Protein determination was performed according to the method of Bradford (22).
Glutathione Conjugation of Aminochrome

Synthesis of Aminochrome and Related Compounds

Oxidation of dopamine to aminochrome was performed as described previously (4). The oxidizing agent was the Mn"-pyrophosphate complex prepared as described by Archibald and Fridovich (23). Oxidation of 4-S-glutathionyl-5,6-dihydroxyindoline to 4-S-glutathionyl-5,6-dihydroxyindoline was performed as described by Mason (24). Three-hundred micrograms of 12 m HCl was added to 1 ml of 300 μM 4-S-glutathionyl-5,6-dihydroxyindoline solution in 25 mM sodium phosphate, pH 6.5, to decrease the pH to 1.3. The reaction mixture was incubated for 30 min at room temperature. The absorption spectra before and after oxidation of the indole ring to indole were recorded.

Assay Conditions

Conjugation of Aminochrome with Glutathione—The standard assay system for measuring conjugation of aminochrome with GSH catalyzed by GSTs contained 0.1 mM sodium phosphate, pH 6.5, 1 mM GSH, and 300 μM aminochrome at 30 °C. The reaction was started by the addition of GST. The reaction was recorded by monitoring the decrease in aminochrome absorbance at 475 nm. The reaction rate was calculated by using the extinction coefficient of aminochrome of 3058 M⁻¹ cm⁻¹ (4). Aminochrome Reduction Catalyzed by NADPH-Cytochrome P450 Reductase—The assay system of 1 ml contained 50 mM Tris-Cl, pH 6.5, 0.25 M sucrose, 30 μM aminochrome, 1 mM (when present) GSH, 60 μg (when present) of GST M1-1 or 8 μg (when present) of GST M2-2, and 250 μM NADPH. The formation of aminochrome (4) was allowed to proceed for 2 min in the cuvette before the addition of GSH and NADPH. The reaction was started by the addition of GST and 5 μg of purified NADPH-cytochrome P450 reductase at 30 °C. The reaction was monitored by recording NADPH oxidation at 340 nm (using an extinction coefficient of 6220 M⁻¹ cm⁻¹) (4). Oxygen consumption was monitored with an oxygen electrode from Hansatech D. W. (King’s Lynn, United Kingdom).

NMR Studies of the Reaction Product

For the structural analysis 300 μM aminochrome and 1 mM GSH were incubated with 100 μg of GST M1-1 in 100 ml of 0.1 mM sodium phosphate, pH 6.5, for 30 min. The aqueous solution was diluted with 1-butanol and concentrated to 25 ml under reduced pressure. More 1-butanol was added, and the concentration procedure was repeated until all traces of water were removed. The resulting solution was concentrated to 0.5 ml. The residue was mixed with diethyl ether (10 ml), and acetic anhydride (0.5 ml) was added, followed by triethylamine (1 ml) and 4-dimethylaminopyridine (catalytic amount). The resultant mixture gave a clear solution after 10 min and was stirred for a further 2 h, during which time considerable darkening occurred. The reaction mixture was poured into saturated sodium hydrogen carbonate solution (50 ml), extracted with dichloromethane (5 × 50 ml), dried with sodium sulfate, and concentrated. The residue was purified on a silica gel column (0.5 × 20 cm) using a stepwise gradient of ethyl acetate in pentane (0–100%) followed by ethyl acetate (0–30%) as eluent. The major fraction was isolated and analyzed in CDCl₃ solution by NMR spectroscopy. NMR spectra were recorded at 399.78 MHz using a JEOL-JNM400 spectrometer. Standard pulse sequences were used for double quantum-filtered COSY and NOESY (nuclear Overhauser enhancement spectroscopy) spectra; for the latter, mixing times were varied between 100 and 600 ms.

RESULTS

Glutathione Conjugation of Aminochrome—GSH reacts nonenzymatically with aminochrome, forming a conjugate at pH 6.5 and 30 °C (Table I). The enzymatic conjugation of aminochrome with GSH was studied with six different human isoenzymes, GSTs A1-1, A2-2, P1-1, M1-1, M2-2, and M3-3. All these GSTs were found to catalyze the conjugation of aminochrome, although major differences in the rates were found. Class Mu GSTs exhibited the highest specific activity, in particular GST M2-2 (148 μmol/min/mg of enzyme), while other GSTs had much lower specific activities (Table I). The activity of GST M2-2 with aminochrome was comparable to that with 1-chloro-2,4-dinitrobenzene, which usually is by far the most active substrate for GSTs. The conjugation of aminochrome was recorded by following the decrease in the absorbance at 475 nm, which is the typical quinone absorption peak (Fig. 1, A and B). A linear correlation between initial velocity and amount of enzyme was demonstrated with GSTs M1-1 and M2-2 (Fig. 2).

Characterization of the Aminochrome Conjugate—The decrease in the absorbance at 475 nm in the visible region caused by conjugation of aminochrome with GSH was also accompanied by a decrease in the absorbance of aminochrome at 298 nm in the ultraviolet region (Fig. 1). The decrease in aminochrome absorbance at 475 nm suggests that GSTs M1-1 and M2-2 catalyze a conjugation, which can be regarded as a two-electron reduction of aminochrome. The absorption spectrum of the glutathione conjugate exhibited two peaks at 277 and 295 nm. However, to determine whether the decrease in the absorbance at 475 nm was due to the reduction of aminochrome or was a consequence of the addition of glutathione to the carbonyl system for measuring conjugation of aminochrome with GSH catalyzed by GSTs contained 0.1 mM sodium phosphate, pH 6.5, 1 mM GSH, and 300 μM aminochrome at 30 °C. The reaction was started by the addition of GST. The reaction was recorded by monitoring the decrease in aminochrome absorption at 475 nm. The reaction rate was calculated by using the extinction coefficient of aminochrome of 3058 M⁻¹ cm⁻¹ (4).

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groups, a potent oxidizing agent was used to oxidize leucoaminochrome-GSH to the corresponding o-quinone. The addition of the Mn$^{3+}$-pyrophosphate complex to leucoaminochrome-GSH resulted in its oxidation to aminochrome-GSH with a new absorption peak in the visible region at 620 nm, supporting the view that the addition of glutathione does not affect the o-quinone structure (Fig. 3A). In the ultraviolet region, a new peak at 310 nm was also observed (data not shown).

NMR studies were performed to determine the structure of the glutathione conjugate. However, direct NMR spectroscopic observation of the product was not possible since concentration of the reaction solution resulted in polymerization. Azeotropic removal of the water with 1-butanol under reduced pressure followed by acylation of the concentrate afforded the crude tetraacetate (Fig. 4A). NMR spectroscopy of the product showed coupled doublets at 6.64 and 7.4 ppm, consistent with oxidation of the dihydroxyindoline followed by isomerization to the corresponding indole, probably occurring during the workup procedure. The structure of the product was confirmed by the presence of nuclear Overhauser effect correlations between H-7 and two acetate groups and between H-3 and the CH$_2$ of the cysteine residue (Fig. 4B). We found that the structure of the acylated conjugate was 4-S-[acetylglutamoyl]-N-cysteinyl-N-glycynl]-5,6-diacetoxyindole: $^1$H NMR δ (ppm) 1.88 (2H, dt, $J = 7.0$, 8.0 Hz, β-CH$_2$-Glu), 2.20–2.30 (14H, m, γ-CH$_2$-Glu, CH$_3$-acetyl), 3.08 (2H, m, CH$_2$-Cys), 3.40–3.44 (2H, m (AB), CH$_2$-Gly), 3.76 (1H, t, $J = 8.0$ Hz, CH-Glu), 4.16 (1H, m, CH-Cys), 6.64 (1H, d, $J = 3.0$ Hz, H-3), 7.10, (1H, s, H-7), and 7.45 (1H, d, $J = 3.0$ Hz, H-2). The data obtained were consistent with the data for the nonacylated compound previously isolated (25). Therefore, we conclude that the structure of the nonacylated conjugate is 4-S-glutathionyl-5,6-dihydroxyindole. Acetylation of 4-S-glutathionyl-5,6-dihydroxyindole to block the free hydroxyl and amino groups accompanied by oxidation and isomerization of indoline to indole. Acetylation was performed as described under "Experimental Procedures." B, nuclear Overhauser effect correlations used for structural assignment of the indole-glutathione conjugate by NMR analysis.
obtained after oxidation of indoline to indole under acid catalysis according to Mason (24). The two peaks of the conjugate at 277 and 295 nm did change after acid catalysis to 295 and 307 nm, a result in agreement with the data reported by D'Ischia et al. (25) (data not shown). Therefore, we conclude that the structure of the conjugate is 4-S-glutathionyl-5,6-dihydroxyindole, which was subsequently oxidized to the indole derivative during the workup and acylation procedures.

An important question was whether the glutathione conjugate could be oxidized by molecular oxygen like reduced forms of aminochrome (o-semiquinone and o-hydroquinone). Fig. 3B shows the absorption spectra of the aminochrome (spectrum a) and 4-S-glutathionyl-5,6-dihydroxyindole at 15, 30, and 60 min (spectra b–d) in the visible region. However, no increase in the absorbance at 620 nm, where the o-quinoine has an absorption maximum, was observed even after 60 min (spectrum d), suggesting that 4-S-glutathionyl-5,6-dihydroxyindole is stable in the presence of oxygen. These results were in agreement with the fact that no oxygen consumption was measurable during conjugation of aminochrome to 4-S-glutathionyl-5,6-dihydroxyindole or after completion of the reaction (data not shown).

Superoxide radicals (O$_2^-$) have been reported to play a major role in the reoxidation of leucoaminochrome produced by the NAD(P)H:quinone oxidoreductase-catalyzed reduction of aminochrome (9). Therefore, we have studied the possibility that superoxide radicals may catalyze the autoxidation of 4-S-glutathionyl-5,6-dihydroxyindole by using xanthine oxidase and hypoxanthine to produce superoxide radicals. However, no oxidation of 4-S-glutathionyl-5,6-dihydroxyindole in the presence of superoxide radicals was observed. The possibility that other biological oxidizing agents such as H$_2$O$_2$ may oxidize the conjugate was also studied. However, no autoxidation of 4-S-glutathionyl-5,6-dihydroxyindole was observed in the presence of H$_2$O$_2$ (data not shown).

GSTs M2-2 and M1-1 Compete with NADPH-Cytochrome P450 Reductase in the Reduction of Aminochrome—One-electron reduction of aminochrome catalyzed by enzymes such as NADPH-cytochrome P450 reductase has been postulated to be responsible for the formation of reactive oxygen species that promote neurodegenerative processes in the dopaminergic system (9). Therefore, it is of interest to know whether GST M2-2 and other GSTs may compete with NADPH-cytochrome P450 reductase in the metabolism of aminochrome. Fig. 5 shows that the reduction of aminochrome by NADPH-cytochrome P450 reductase is accompanied by a constant rate of NADPH oxidation (21 nmol of NADPH/min) recorded at 340 nm. The addition of 1 mM GSH to the incubation mixture resulted in 31% inhibition of autoxidation. However, the addition of GST M1-1 (60 

**FIG. 5.** Glutathione transferases M1-1 (A) and M2-2 (B) compete with NADPH-cytochrome P450 reductase in the reduction of aminochrome. The incubation conditions are described under "Experimental Procedures." The values are the means ± S.D. of three experiments.

The results presented in this paper demonstrate that human GSTs catalyze the reductive conjugation of aminochrome to 4-S-glutathionyl-5,6-dihydroxyindole (Scheme 1). The NMR studies showed that the structure of the acetylated derivative produced for analysis was 4-S-[(acetylglutamoyl)-N-cysteinyl-N-glycinyl]-5,6-diacetoxyindole. We conclude that the structure of the product of the GST-catalyzed reaction is 4-S-glutathionyl-5,6-dihydroxyindole since the spectrum of the corresponding oxidized (nonacetylated) compound, 4-S-glutathionyl-5,6-dihydroxyindole, is not the same as the conjugate (4-S-glutathionyl-5,6-dihydroxyindole) enzymatically produced. The two peaks of 4-S-glutathionyleucoaminochrome at 278 and 295 nm are shifted to 295 and 307 nm in 4-S-glutathionyl-5,6-dihydroxyindole due to oxidation of the dihydroxyindole followed by acid-catalyzed isomerization to indole during the workup and acylation procedures required for NMR analysis.

GSTs are known to have broad and overlapping substrate specificities, but only a few of their numerous substrates are so markedly preferentially used by one isoenzyme as aminochrome. With this substrate, GST M2-2 has a specific activity >200-fold above that of any other GST (Table 1). Another example is 2-cyano-1,3-dimethyl-1-nitrosoguanidine, for which GST M2-2 is also the most active enzyme by almost 2 orders of magnitude (26). The finding that aminochrome is an excellent GST substrate extends the number of physiologically occurring compounds that serve as substrates for GSTs. Aminochrome is a product of oxidative metabolism, like many other pathophysio logically relevant GST substrates such as alkenals and hydroperoxides generated by biological oxidations and radical reactions (27).
NADPH-cytochrome P450 reductase and NADPH:quinone oxidoreductase catalyze one- and two-electron reduction of aminochrome to o-semiquinone and leucoaminochrome (o-hydroquinone), respectively. These products are oxidized by $O_2$, yielding reactive oxygen species. However, a major difference in the rate of oxidation of o-semiquinone and o-hydroquinone forms has been observed (4, 9). The autoxidation of o-semiquinone is too fast to be recorded by conventional steady-state kinetics, whereas the autoxidation of the o-hydroquinone can readily be monitored by the decrease in the quinone absorbance peak at 475 nm. The lability of o-semiquinone and leucoaminochrome in the presence of oxygen contrasts with the chemical stability of 4-S-glutathionyl-5,6-dihydroxyindoxylidine since no oxidation was observed during conjugation or after the completion of the reaction. The reactivity of 4-S-glutathionyl-5,6-dihydroxyindoxylidine was found to be completely different from that of leucoaminochrome and o-semiquinone. Molecular oxygen ($O_2$) is mainly responsible for oxidation of o-semiquinone (91% of the total oxygen-dependent autooxidation) (9), while superoxide radicals are mainly responsible for oxidation of the leucoaminochrome (89% of the total oxygen-dependent autooxidation) (4, 9). However, superoxide radicals, hydrogen peroxide, and molecular oxygen are not able to oxidize 4-S-glutathionyl-5,6-dihydroxyindoxylidine under the conditions investigated.

It seems reasonable to consider glutathione conjugation of aminochrome as a reaction terminating any possible redox cycling since it prevents the formation of reactive oxygen species during one- or two-electron reduction of aminochrome. It is obvious that a very small amount of aminochrome can give rise to a large production of reactive oxygen species due to the redox cycling properties of aminochrome, emphasizing the importance of the glutathione conjugation. Our results show that GST M2-2, as well as GST M1-1, by a competing conjugation reaction prevents autooxidation otherwise elicited by the reduction of aminochrome catalyzed by NADPH-cytochrome P450 reductase (Fig. 5). One-electron reduction of aminochrome catalyzed by NADPH-cytochrome P450 reductase has been postulated to be the mechanism of formation of reactive oxygen species, which can cause neurodegeneration that in the nigrostriatal system may result in Parkinson’s disease or in the mesolimbic system in schizophrenia (9).

The glutathione conjugation catalyzed by class Mu GSTs seems to be the most important of the reported antioxidant reactions related to aminochrome metabolism since this reaction prevents the redox cycling between oxidized and reduced forms of aminochrome as a consequence of the chemical stability of 4-S-glutathionyl-5,6-dihydroxyindoxylidine. NAD(P)H:quinone oxidoreductase has also been postulated to provide an important cellular defense against formation of reactive oxygen species since the reaction catalyzed by NAD(P)H:quinone oxidoreductase competes with one-electron donors such as NADPH-cytochrome P450 reductase for the reduction of aminochrome to leucoaminochrome (4). However, the antioxidant role of NAD(P)H:quinone oxidoreductase is dependent on the presence of superoxide dismutase, catalase, or GSH peroxidase, which inhibit leucoaminochrome autoxidation, or on the action of conjugation enzymes such as sulfotransferase or catechol ortho-methyltransferase. It seems plausible that human subjects with low expression or lack of the genes coding for NAD(P)H:quinone oxidoreductase, GST M1-1, and/or GST M2-2 will be at higher risk for neurodegeneration of the dopaminergic nigrostriatal or mesolimbic system, which may result in the development of Parkinson’s disease and schizophrenia, respectively.

We conclude that GST M2-2 efficiently catalyzes the conjugation of aminochrome, thus preventing the formation of reactive oxygen species. Other GSTs, such as the class Mu enzymes GST M1-1 and GST M3-3, also promote this conjugation reaction, albeit with much lower catalytic activities. We cannot disregard the possibility that other enzymes not yet tested also play a role in detoxication of aminochrome. An example is GST M5-5, which has been cloned from a cDNA library from the frontal cortex of human brain (28). To establish the physiological relevance of the GST activity to neurodegenerative diseases such as Parkinson’s disease and schizophrenia, it is necessary to explore the regional distribution of GST M2-2 and other class Mu GSTs in the brain and in the dopaminergic system.

REFERENCES

1. Graham, D. G. (1978) Mol. Pharmacol. 14, 633–643
2. Hawley, M. D., Tatawawadi, S. V., Piekarski, S., and Adams, R. N. (1967) J. Am. Chem. Soc. 89, 447–450
3. Harrison, W. H., Whisler, W. W., and Hill, B. J. (1968) Biochemistry 7, 3089–3093
4. Segura-Aguilar, J., and Lind, C. (1989) Chem. Biol. Interact. 72, 309–324
5. Baez, S., Linderson, Y., and Segura-Aguilar, J. (1994) Chem. Biol. Interact. 93, 103–116
6. Linderson, Y., Baez, S., and Segura-Aguilar, J. (1994) Biochem. Biophys. Acta 1200, 197–204
7. Baez, S., and Segura-Aguilar, J. (1994) Redox Report 1, 65–70
8. Baez, S., and Segura-Aguilar, J. (1995) Biochem. Mol. Med. 56, 37–44
9. Baez, S., Linderson, Y., and Segura-Aguilar, J. (1995) Biochem. Mol. Med. 54, 12–18
10. Graham, D. G. (1984) Neurotoxicology (Little Rock 5, 83–96
11. Cadet, J. L., and Kahler, L. A. (1994) Neurosci. Biobehav. Rev. 18, 457–467
12. Smythies, J. (1996) Proc. R. Soc. Lond. B Biol. Sci. 263, 487–489
13. Hayes, J. D., and Pulford, D. J. (1995) Crit. Rev. Biochem. Mol. Biol. Mol. Biol. 30, 445–600
14. Segura-Aguilar, J., Jönsson, K., Tiderfelt, U., and Paul, C. (1992) Leuk. Res. 16, 631–637
15. Segura-Aguilar, J., Cortez-Vicario, L., Llombart-Bosch, A., Ernster, L., Monsalve, E., and Romero, F. J. (1990) Carcinogenesis 11, 1727–1732
16. Segura-Aguilar, J., Jönsson, K., Tiderfelt, U., and Paul, C. (1992) Leuk. Res. 16, 631–637
17. Beyer, R. E. (1992) Biochem. Cell Biol. 70, 390–403
18. Beyer, R. E., Segura-Aguilar, J., Di Bernardo, S., Cavazzoni, M., Fato, R., Fiorentini, D., Cristina Galli, M., Setti, M., Landi, L., and Lenaz, G. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 2528–2532
19. Mannervik, B., Awasthi, Y. C., Board, P. G., Hayes, J. D., Di Ilio, C., Ketterer, B., Listowsky, I., Morgenstern, R., Muramatsu, M., Pearson, W. R., Pickett, C. B., Sato, K., Widersten, M., and Wolf, C. R. (1992) Biochem. J. 292, 305–306
20. Mannervik, B., and Guthenberg, C. (1981) Methods Enzymol. 77, 231–235
21. Yasukochi, Y., and Masters, B. S. S. (1976) J. Biol. Chem. 251, 5337–5344
22. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
23. Archibald, F. S., and Fridovich, I. (1982) Arch. Biochem. Biophys. 214, 452–463
24. Mason, H. S. (1948) J. Biol. Chem. 172, 83–99
25. D’Ischia, A., Napolitano, A., and Prota, G. (1987) Tetrahedron 43, 5351–5356
26. Mannervik, B., and Widersten, M. (1995) in Advances in Drug Metabolism in Man (Pacifici, G. M., and Frachia, G. N., eds.) pp. 407–459, European Commission, Luxembourg, Belgium
27. Berhane, K., Widersten, M., Engström, Å., Koazrich, J. W., and Mannervik, B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1480–1484
28. Takahashi, Y., Campbell, E. A., Hiraiz, V., Takayama, T., and Listowsky, I. (1993) J. Biol. Chem. 268, 8893–8898
29. Habig, W. H., Pabst, M. J., and Jakoby, W. B. (1974) J. Biol. Chem. 249, 7130–7139

\[ \text{Scheme 1} \]

\[ \text{GSH} \]

\[ \text{GST M2-2} \]