Activation of Rat Liver Microsomal Glutathione S-Transferase by Reduced Oxygen Species*

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(Received for publication, August 12, 1988)

The effect of enzymatically generated reduced oxygen metabolites on the activity of hepatic microsomal glutathione S-transferase activity was studied to explore possible physiological regulatory mechanisms of the enzyme. Noradrenaline and the microsomal cytochrome P-450-dependent monooxygenase system were used to generate reduced oxygen species. When noradrenaline (>0.1 mM) was incubated with rat liver microsomes in phosphate buffer (pH 7.4), an increase in microsomal glutathione S-transferase activity was observed, and this activation was potentiated in the presence of a NADPH-generating system; the glutathione S-transferase activity was increased to 180% of the control with 1 mM noradrenaline and to 400% with both noradrenaline and NADPH. Superoxide dismutase and catalase inhibited partially the noradrenaline-dependent activation of the enzyme. In the presence of diethyhydrotoxic and glutathione, the activation of the glutathione S-transferase by noradrenaline, with or without NADPH, was not observed. In addition, the activation of glutathione S-transferase activity by noradrenaline and glutathione disulfide was not additive when both compounds were incubated together. These results indicate that the microsomal glutathione S-transferase is activated by reduced oxygen species, such as superoxide anion and hydrogen peroxide. Thus, metabolic processes that generate high concentrations of reduced oxygen species may activate the microsomal glutathione S-transferase, presumably by the oxidation of the sulfhydryl group of the enzyme, and this increased catalytic activity may help protect cells from oxidant-induced damage.

Glutathione S-transferases (EC 2.5.1.18) catalyze the reaction of glutathione with many xenobiotics and their reactive electrophilic metabolites (1, 2). Rat liver contains large amounts of cytosolic and microsomal glutathione S-transferase activity, and both have been isolated and characterized (1, 3). Microsomal glutathione S-transferase amounts to 3.1% of the total microsomal protein in rats (4) and contains one cysteine residue/subunit (5, 6). In contrast to the cytosolic glutathione S-transferases, microsomal transferase activity is low with many substrates and is activated by the covalent modification of the sulfhydryl group (7, 8), by limited proteolysis (9), by radiation (10), or by mixed disulfide formation (11, 12). Because microsomal cytochromes P-450 catalyze the transformation of xenobiotics to reactive metabolites, the microsomal glutathione S-transferase may be more important than the cytosolic glutathione S-transferases in the detoxication of such reactive metabolites. It is of importance, therefore, to understand the mechanism of activation of the microsomal glutathione S-transferase. Masukawa and Iwata (11) reported that the glutathione-depleting agent phenone increases both protein-mixed disulfide formation and microsomal glutathione S-transferase activity. Thus, mixed disulfide bond formation between the microsomal transferase and disulfides, such as glutathione disulfide, may be involved in the physiological regulation of enzyme activity. Moreover, because cytochromes P-450 and NADPH-cytochrome P-450 reductase generate reduced oxygen species (13–18), the microsomal glutathione S-transferase may also be activated by oxidants produced in the liver.

In the present study, we investigated the activation of the microsomal glutathione S-transferase by enzymatically generated oxidants. Because the oxidation of catecholamines generates reduced oxygen metabolites (19–22), we determined whether the microsomal glutathione S-transferase is activated by noradrenaline in the presence or absence of a NADPH-generating system. The results show that the microsomal glutathione S-transferase is activated by reduced oxygen species, which are formed by noradrenaline oxidation or by cytochromes P-450.

MATERIALS AND METHODS

Noradrenaline, catalase, superoxide dismutase, NADP*, NADPH, glucose 6-phosphate, glutathione, and glutathione disulfide were purchased from Sigma. 1-Chloro-2,4-dinitrobenzene was obtained from Wako Pure Chemical Industries, Ltd., Tokyo. Glucose-6-phosphate dehydrogenase was purchased from Oriental Yeast Ltd., Tokyo. All other reagents used were of analytical grade.

Liver microsomes were prepared from male Sprague-Dawley rats (200–300 g). The rats were starved overnight and were killed by decapitation; the liver was removed after perfusion in situ with ice-cold 1.15% potassium chloride solution. The liver was homogenized with 2 volumes of the same solution and centrifuged at 9,000 × g for 30 min. The supernatant was centrifuged at 105,000 × g for 60 min. The resultant microsomal pellet was suspended in 0.15 M Tris-HCl buffer (pH 8.0) and was isolated twice more by centrifugation. The microsomes thus prepared were used immediately or within 1 week after storage at −60 °C. The pellet was resuspended in 0.05 M potassium phosphate buffer (pH 7.4) containing 0.3 mM EDTA and 0.25 M sucrose.

The activation of microsomal glutathione S-transferase activity was measured as follows. Microsomes (0.5–1.2 mg of protein/ml) were incubated with noradrenaline at 37 °C in the presence or absence of various reagents and a NADPH-generating system in 0.05 M potassium phosphate buffer (pH 7.4), and 200-μl samples were removed at the specified times for the assay of glutathione S-transferase activity.
Microsomal Glutathione S-Transferase

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FIG. 1. Effect of catecholamines on microsomal glutathione S-transferase activity. Microsomes were incubated in the absence (○) or presence of 10 mM L-adrenaline (●) or noradrenaline (▲) at 37 °C, and samples were removed at each time point for measurement of glutathione S-transferase activity (GST) as described under "Materials and Methods." The activities are presented as the mean of duplicate experiments.

FIG. 2. Noradrenaline-dependent activation of microsomal glutathione S-transferase activity in the presence of a NADPH-generating system. Microsomes were incubated in the absence (○) or presence (▲) of 0.1 mM noradrenaline, a NADPH-generating system (●), or both (▲), at 37 °C and samples were removed for assay of the glutathione S-transferase activity (GST) as described under "Materials and Methods."

The NADPH-generating system consisted of 0.33 mM NADP+, 8 mM glucose 6-phosphate, 6 mM MgCl₂, and 0.2 units of glucose 6-phosphate dehydrogenase. All reagents added to the incubation mixture were dissolved in 0.05 M potassium phosphate buffer (pH 7.4). Glutathione S-transferase activity was measured by the method of Habig et al. (23) with 1 mM 1-chloro-2,4-dinitrobenzene and 5 mM glutathione as substrates. Protein concentrations were measured by the method of Lowry et al. (24).

RESULTS

Fig. 1 shows the effect of catecholamines on microsomal glutathione S-transferase activity. Both noradrenaline and adrenaline (10 mM) increased transferase activity, but the effect of noradrenaline was greater than that of adrenaline. As shown in Fig. 2, 0.1 mM noradrenaline alone had little effect on microsomal glutathione S-transferase activity but markedly stimulated enzyme activity in the presence of a NADPH-generating system. Microsomal glutathione S-transferase activity was increased 132% by noradrenaline alone, 260% by a NADPH-generating system, and 400% by the combination of both noradrenaline and a NADPH-generating system. The effect of noradrenaline concentration on microsomal glutathione S-transferase activity is presented in Fig. 3. In the presence or absence of a NADPH-generating system, noradrenaline (>0.1 mM) stimulated microsomal glutathione S-transferase activity.

Microsomal glutathione S-transferase was activated by glutathione disulfide, but the effect of noradrenaline on transferase activity was not additive with the glutathione disulfide-dependent activation of the enzyme (Fig. 4). Noradrenaline (1 mM) in the absence or presence of a NADPH-generating system increased microsomal glutathione S-transferase activity by about 190 and 400%, respectively, and this stimulation was reversed by the addition of dithio-
enzyme thiol and is reversed by diethiothreitol and glutathione. In order to clarify the role of reduced oxygen species in the noradrenaline-dependent activation of microsomal glutathione S-transferase activity, the effect of catalase or superoxide dismutase was studied (Table III). The microsomal glutathione S-transferase activity was increased 167% by 1 mM noradrenaline alone and 128 and 159% by combination with catalase or superoxide dismutase, respectively. In the presence of a NADPH-generating system, noradrenaline increased microsomal glutathione S-transferase activity by 281%, and the activity was decreased to 314% by catalase, to 215% by superoxide dismutase, and to 146% by combination of both catalase and superoxide dismutase. The NADPH-generating system alone also increased microsomal glutathione S-transferase activity, but this effect was little affected by catalase or superoxide dismutase. As shown in Table IV, mannitol did not prevent the activation of microsomal glutathione S-transferase activity by noradrenaline. These data indicate that the microsomal glutathione S-transferase was activated by reduced oxygen metabolites such as hydrogen peroxide or superoxide anion, but not by hydroxyl radical.

Catecholamines are oxidized to adrenochrome and to oxidized products, which absorb at about 480 and 310 nm and at 340 nm, respectively (21). Because glutathione S-transferase activity was measured as the change in absorbance at 340 nm caused by the formation of the glutathione conjugate of 1-chloro-2,4-dinitrobenzene, it was important to determine that oxidized products of catecholamines did not interfere with the measurement of transferase activity. In order to distinguish enzyme activity from the formation of oxidized products of catecholamines, the microsomes were separated by centrifugation after treatment with noradrenaline in the presence of a NADPH-generating system, and the activities of the microsomal and supernatant fractions were measured. Although the supernatant contained substances that absorbed at 480 and 340 nm, no time-dependent change in absorbance at 340 nm was observed when the substrates for the glutathione S-transferase were added (data not shown). Furthermore, the noradrenaline-treated microsomes showed a 3-fold increase in glutathione S-transferase activity compared to the activity of nontreated microsomes (data not shown). These data demonstrate that noradrenaline-activated microsomal glutathione S-transferase activity was present.

**DISCUSSION**

In the present study, it was demonstrated that liver microsomal glutathione S-transferase activity is stimulated by noradrenaline with or without a NADPH-generating system. Much evidence has accumulated showing that adrenaline and

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Effect of diethiothreitol on noradrenaline-dependent activation of microsomal glutathione S-transferase activity</th>
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<tbody>
<tr>
<td>Microsomes were incubated at 37 °C for 10 min in the absence or presence of noradrenaline (1 mM), a NADPH-generating system (NADPH), or diethiothreitol (DTT, 10 mM) as indicated in the table. Transferase activity (GST) was measured as described under &quot;Materials and Methods.&quot; Each value represents the mean ± S.D. of two or three incubations.</td>
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<table>
<thead>
<tr>
<th>Addition</th>
<th>NADPH</th>
<th>DTT</th>
<th>GST activity</th>
<th>Percent of control</th>
</tr>
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<td>Control</td>
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</tr>
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<td></td>
<td>+</td>
<td>−</td>
<td>0.123 ± 0.013</td>
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</tr>
<tr>
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<td>+</td>
<td>0.064 ± 0.006</td>
<td>79</td>
</tr>
<tr>
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<td>−</td>
<td>+</td>
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<td>90</td>
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<td>Noradrenaline</td>
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<td>+</td>
<td>0.296 ± 0.005</td>
<td>365</td>
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<td>0.057 ± 0.004</td>
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<tr>
<th>TABLE II</th>
<th>Effect of glutathione on noradrenaline-dependent activation of microsomal glutathione S-transferase activity</th>
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<tr>
<td>Microsomes were incubated at 37 °C for 10 min in the absence or presence of noradrenaline (1 mM), a NADPH-generating system (NADPH), or glutathione (GSH, 1 mM) as indicated in the table. Transferase activity (GST) was measured as described under &quot;Materials and Methods.&quot; Each value represents the mean ± S.D. of two or three incubations.</td>
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<table>
<thead>
<tr>
<th>Addition</th>
<th>NADPH</th>
<th>GSH</th>
<th>GST activity</th>
<th>Percent of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>0.070 ± 0.007</td>
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<tr>
<td></td>
<td>−</td>
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<td>0.069 ± 0.0</td>
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<tr>
<td>Noradrenaline</td>
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<td>198</td>
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<td>−</td>
<td>0.068 ± 0.001</td>
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<td>0.289 ± 0.003</td>
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<td>0.043 ± 0.005</td>
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<th>TABLE III</th>
<th>Effect of catalase and superoxide dismutase on noradrenaline-dependent activation of microsomal glutathione S-transferase activity</th>
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<td>Microsomes were incubated at 37 °C for 10 min in the absence or presence of noradrenaline (1 mM), a NADPH-generating system (NADPH), catalase (75 μg/ml), or superoxide dismutase (SOD, 15 μg/ml) as indicated in the table. Transferase activity (GST) was measured as described under &quot;Materials and Methods.&quot; Values represent the mean ± S.D. of two to nine incubations.</td>
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<tr>
<th>Addition</th>
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<th>Catalase</th>
<th>SOD</th>
<th>GST activity</th>
<th>Percent of control</th>
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<td>−</td>
<td>−</td>
<td>0.120 ± 0.011</td>
<td>146 ± 13</td>
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<td>0.108 ± 0.009</td>
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<td>0.114 ± 0.017</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>0.104 ± 0.013</td>
<td>128 ± 15</td>
</tr>
<tr>
<td>Noradrenaline</td>
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<td>−</td>
<td>0.157 ± 0.013</td>
<td>167 ± 15</td>
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<tr>
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<td>−</td>
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<td>128 ± 10</td>
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<td>0.258 ± 0.057</td>
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<td>+</td>
<td>0.177 ± 0.009</td>
<td>215 ± 11</td>
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<td>0.120 ± 0.004</td>
<td>146 ± 5</td>
</tr>
</tbody>
</table>

threitol or glutathione (Tables I and II). These results indicate that the noradrenaline-dependent activation of microsomal glutathione S-transferase activity is due to oxidation of the
noradrenaline generate reduced oxygen metabolites during their oxidation (19-22, 25). Thus, noradrenaline may stimulate microsomal glutathione S-transferase activity by the production of reduced oxygen species. Microsomal cytochromes P-450 and NADPH-cytochrome P-450 reductase also generate reduced oxygen metabolites such as superoxide anion or hydrogen peroxide (13-18).

Hence, microsomal glutathione S-transferase activity was enhanced by incubation with a NADPH-generating system alone, and an additional increase in activity was observed when noradrenaline was incubated with a NADPH-generating system. Such activation of the microsomal glutathione S-transferase by noradrenaline or by the combination of noradrenaline and a NADPH-generating system was partially inhibited by catalase and superoxide dismutase, indicating the involvement of hydrogen peroxide and superoxide anion in the activation of microsomal glutathione S-transferase activity. Cytochromes P-450 or NADPH-cytochrome P-450 reductase may transfer electrons to dioxygen resulting in the generation of reduced oxygen species, such as superoxide anion or hydrogen peroxide (14, 17, 18, 26). It is, therefore, likely that the microsomal glutathione S-transferase is activated, directly or indirectly, by hydrogen peroxide or superoxide anion formed by adrenaline oxidation or the cytochrome P-450 monooxygenase system. Although microsomal cytochrome b₅ forms superoxide radicals (27), incubation of noradrenaline with NADH did not enhance the activation of microsomal glutathione S-transferase activity (data not shown). Mannitol, a hydroxyl radical scavenger (28), did not inhibit the activation of the microsomal glutathione S-transferase by noradrenaline in the presence or absence of a NADPH-generating system, indicating that the hydroxyl radical does not contribute to the activation of the glutathione S-transferase. The potentiation of the effect of noradrenaline on microsomal glutathione S-transferase activity by a NADPH-generating system may indicate that reduced oxygen species formed by the cytochrome P-450-dependent monooxygenase system may oxidize the noradrenaline to the semiquinone, which in turn may generate the superoxide anion radical. Thus, larger amounts of reduced oxygen species may be produced when noradrenaline is incubated with a NADPH-generating system leading to increased activation of microsomal glutathione S-transferase activity.

The effect of noradrenaline on the microsomal glutathione S-transferase was blocked by the addition of dithiothreitol or glutathione. These data indicate that the activation of the microsomal glutathione S-transferase by reduced oxygen species is due to oxidation of the protein sulfhydryl group on the enzyme to the disulfide. As reported earlier (11, 12) glutathione disulfide increases microsomal glutathione S-transferase activity by the formation of mixed disulfides with the sulfhydryl group of the enzyme, and this effect is reversed by glutathione. Thus, oxidation of the single cysteine residue of the enzyme to a disulfide increases microsomal glutathione S-transferase activity and may serve as a regulatory mechanism for the enzyme. This finding supports the concept that oxidative stress, which increases glutathione disulfide concentrations or generates high concentrations of reduced oxygen species, may stimulate the microsomal glutathione S-transferase activity. Furthermore, because the ferricytochrome P-450-oxygen complex itself oxidizes the Ca²⁺ pump (29) and cytochromes P-450 may generate more reduced oxygen species when substrates are present (30), it is proposed that the cytochrome P-450-dependent monooxygenase system may transform xenobiotics to reactive metabolites and simultaneously generate active oxygen species that may oxidize the sulfhydryl group of the microsomal glutathione S-transferase, resulting in the activation of the enzyme. The activation of microsomal glutathione S-transferase may be reversed enzymatically.

The information presented in this report, along with published data, affords an opportunity to speculate about the cellular regulation and role of the microsomal glutathione S-transferase. The formation of reduced oxygen species or glutathione disulfide, or both, which may occur during oxidative stress or during the metabolism of xenobiotics, may serve to activate the microsomal transferase by oxidizing the cysteine thiol to a disulfide. The resulting increased catalytic activity of the transferase may detoxify reactive metabolites of xenobiotics by conjugation with glutathione or may detoxify hydrogen peroxide or organic hydroperoxides by serving as a glutathione peroxidase, a known catalytic function of the microsomal transferase (31). This may explain the observation that carbon tetrachloride, which is well known to initiate microsomal lipid peroxidation, stimulates microsomal glutathione S-transferase activity (32). The activation of the microsomal transferase may be reversed by the microsomal protein disulfide-isomerase (EC 5.3.4.1) (33), whose activity is stimulated by glucose (34). Although the possible regulation of the microsomal transferase by the protein disulfide-isomerase has not been investigated, such studies are warranted because of the known role of reversible oxidation-reduction of enzyme thiols and disulfides in metabolic regulation (35, 36).

REFERENCES