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Regulation of microsomal and cytosolic glutathione *S*-transferase activities by *S*-nitrosylation

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Abstract

There is increasing evidence that *S*-nitrosylation is a mechanism for the regulation of protein function via the modification of critical sulfhydryl groups. The activity of rat liver microsomal glutathione *S*-transferase (GST) is increased after treatment with *N*-ethylmaleimide (NEM), a sulfhydryl alkylating reagent, and is also increased under conditions of oxidative stress. In the present study, preincubation of purified rat liver microsomal GST with *S*-nitrosoglutathione (GSNO) or the nitric oxide (NO) donor, 1,1-diethyl-2-hydroxy-2-nitrosohydrazine (DEA/NO), resulted in a 2-fold increase in enzyme activity. This increase in activity was reversed by dithiothreitol. The initial treatment of microsomal GST with either GSNO or DEA/NO was associated with an 85% loss of free sulfhydryl groups. After removal of the nitrosylating agents over a 6-hr period, approximately 50% of the enzyme was still nitrosylated, as determined by redox chemiluminescence. Furthermore, preincubation of either purified enzyme or hepatic microsomes with GSNO or DEA/NO prevented further enzyme activation by NEM, suggesting that NEM and the NO donors interact with a common population of sulfhydryl groups in the enzyme. In contrast, both NEM and NO donors partially inhibited the activity of cytosolic GST isoforms. The inhibitory activity of NEM and NO donors was much more evident when the GST pi isoform was used instead of a mixture of GST isoforms. These data suggest that there may be differential regulation of microsomal and cytosolic GST activities under conditions of nitrosative stress.

Keywords: *S*-Nitrosylation; Microsomal glutathione *S*-transferase; *S*-Nitrosoglutathione; DEA/NO

1. Introduction

The GSTs are involved in the biotransformation of numerous carcinogenic, mutagenic, toxic, and pharmacologically active compounds [1]. These enzymes are found largely in the cytosol as homodimers or heterodimers, and in the rat over twenty different GST subunits have been identified [2]. A membrane-bound GST that accounts for up to 3% of microsomal protein also has been identified [3,4]. However, this GST isoform bears no obvious structural resemblance (amino acid sequence, molecular weight, or immunological properties) to the cytosolic GSTs. The microsomal GST exists as a trimer of identical 17.2 kDa subunits [5–8]. Unlike the cytosolic GSTs, microsomal GST activity is increased by partial proteolysis [9] or by sulfhydryl reagents, such as NEM, that bind covalently to the sole cysteine residue (Cys49) in each polypeptide [10]. This modification results in a 10- to 15-fold increase in enzyme activity. In addition, the redox state of sulfhydryl groups in microsomal GST can also regulate enzyme activity.

NO is an important signaling molecule in both physiological and pathological processes. *S*-Nitrosothiols, such as GSNO, may function as storage forms of NO *in vivo*, and may participate in transnitrosation reactions. It is becoming increasingly evident that *S*-nitrosylation is a mechanism for modifying protein function through alterations in the function of sulfhydryl groups. *S*-Nitrosylation of a variety of proteins has been demonstrated, ranging from ion channels, enzymes, transcription factors, G-proteins, and kinases. Since modification of the sulfhydryl group in microsomal GST can regulate its activity, we hypothesized that this enzyme could be another protein target of NO. On

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Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; DEA/NO, 1,1-diethyl-2-hydroxy-2-nitrosohydrazine, sodium salt; DTNB, 5,5′-dithio-bis (2-nitrobenzoate); DTT, dithiothreitol; GSH, glutathione; GSNO, *S*-nitrosoglutathione; GST, glutathione *S*-transferase; NEM, *N*-ethylmaleimide; NO, nitric oxide.

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the other hand, it has been reported that GSNO inhibits cytosolic GST activity by competing with GSH for binding at the active site of the enzyme, rather than by covalent modification of the enzyme [11]. In this work, we investigated the effect of NO donors on rat liver microsomal and cytosolic GST activity by using NEM to monitor the state of sulfhydryl groups in the enzyme.

2. Materials and methods

2.1. Purification of rat liver microsomal GST

Hepatic microsomes were prepared from male Sprague–Dawley rats (300–350 g) as described [12] and then washed twice with 0.15 M Tris/HCl, pH 8.0, to decrease cytosolic contamination. The enzyme was purified using hydroxyapatite and CM-Sepharose chromatography as described [13], with the exception that treatment of the microsomes with NEM was omitted. Protein in the purified enzyme preparation migrated as a single band at about 17 kDa on reducing SDS–PAGE gels (Fig. 1). Human GST pi, purified from placenta, was obtained from the Sigma Chemical Co. Rat hepatic GSTs were obtained from Sigma as a mixture of cytosolic isoforms. There was no evidence of contamination by microsomal GST in this enzyme preparation (Fig. 1). Protein content of samples was determined by the method of Bradford [14] using BSA as the standard.

2.2. Synthesis of GSNO

GSNO was prepared as described previously [15] by reacting equimolar amounts of sodium nitrite and GSH. Briefly, 100 mM $NaNO_2$ was mixed with 100 mM HCl-acidified GSH for 5 min and then neutralized with 1.0 N NaOH. The concentration of GSNO was determined spectrophotometrically at 334 nm ($\epsilon = 767\ M^{-1}\ cm^{-1}$). All procedures were performed at 4°.

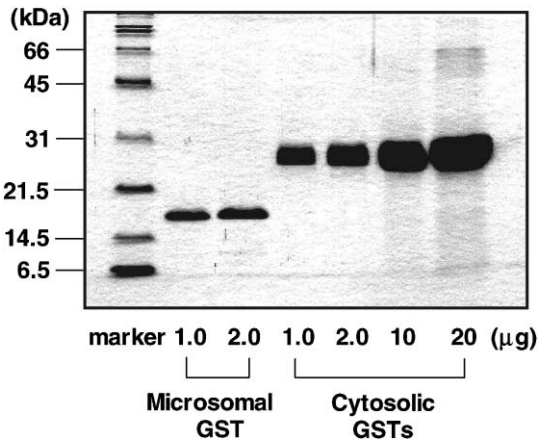

Fig. 1. SDS–PAGE of microsomal and cytosolic GST preparations. The indicated amounts of purified microsomal and cytosolic GSTs were resolved on a 15% SDS–PAGE gel and stained with Coomassie Blue. For the microsomal GST, only one protein band (about 17 kDa) was identified. For the commercial cytosolic GST preparation, contamination with microsomal GST was not evident.

2.3. Treatment of microsomal and cytosolic GSTs with NO donors

Prior to experiments, GSH in both purified cytosolic and microsomal GST preparations (maximum volume, 0.4 mL) was removed by dialysis against four changes of 100 mL of 10 mM Tris/HCl (pH 7.8), or 10 mM potassium phosphate (pH 7.0) containing 0.1 mM EDTA, 1% Triton X-100, and 20% glycerol, respectively. Cytosolic or microsomal GST (2 μg) was incubated with 1.0 mM GSNO or 0.5 mM DEA/NO (Calbiochem) in 100 mM potassium phosphate buffer, pH 7.0, at 37° for 10 min, in a total volume of 100 μL. Some samples were then incubated with 1.0 mM DTT for a further 20 min. In the experiments examining the effect of treatment with NO donors on enzyme activation by NEM, 3.0 mM NEM was added subsequent to reduction of the enzyme by DTT, and then incubated at room temperature for 1.0 min prior to determination of enzyme activity.

2.4. Determination of protein S-nitrosothiol content

For determination of protein *S*-nitrosylation by GSNO, microsomal GST (300–400 μg/mL) was incubated with 1.0 mM GSNO at 37° for 10 min. An aliquot was washed extensively with 100 mM potassium phosphate buffer using a Microcon® centrifugal filter device (Millipore), until the theoretical concentration of GSNO was reduced to between 0.5 and 2 μM. Aliquots of this sample were used for the assay of protein *S*-nitrosothiol content and for protein determination. For determination of protein *S*-nitrosylation by DEA/NO, microsomal GST (50–60 μg/mL) was incubated with 0.5 mM DEA/NO at 37° for 10 min, and then placed on ice for 5–6 hr to allow for the release and dissipation of the remaining NO from DEA/NO. Aliquots of the sample were then used for the determination of protein *S*-nitrosothiol content. In parallel with the above, additional aliquots of the microsomal GST samples were taken prior to and after incubation with GSNO or DEA/NO for determination of the free SH content of the enzyme using Ellman's reagent (DTNB). In all experiments, control incubations were performed in which protein was absent. As described by Noble and Williams [16], NO release from nitrosylated microsomal GST was achieved by transnitrosation of the NO moiety from the protein to cysteine to form *S*-nitrosocysteine, followed by Cu^{2+}-catalyzed decomposition of the latter. The NO released was then quantitated using a chemiluminescence detector (Sievers Research Inc., model 207B), using the general method of Brien *et al.* [17]. Protein samples were first deoxygenated by purging the headspace with nitrogen for at least 10 min. The sample was then added to a sealed

flask containing deoxygenated 100 mM potassium phosphate buffer, pH 7.4, 1.0 mM L-cysteine, and 0.2 mM $CuSO_4$. The final protein content of the samples was 150–300 nM. The samples were incubated for 5 min at 37°, and 40 μL of the headspace gas was removed and injected into the chemiluminescence detector. NO was quantitated by comparison with a standard curve prepared using solutions of GSNO (50–400 nM final concentration) treated in an identical manner.

2.5. Enzyme assay

GST activity was determined by the method of Habig *et al.* [18]. The assay was performed in 0.1 M potassium phosphate buffer, pH 6.5, at 25° using 1.0 mM GSH and 1.0 mM CDNB as substrates. The assay buffer for microsomal GST activity also contained 0.5% Triton X-100. Data are presented as the means ± SD of replicate measurements from a single enzyme preparation.

3. Results

On reducing SDS–PAGE gels (Fig. 1), the microsomal GST, purified from rat liver, migrates as a single band at about 17 kDa, although in the native state the enzyme is thought to exist as a homotrimer [5–8]. The specific activity of the undialyzed, purified enzyme was 1.6 μmol/min/mg protein, and this was increased about 13-fold after treatment with NEM. A similar fold increase in activity after NEM treatment was observed using the dialyzed enzyme preparation. These data are in good agreement with those of other investigators [13,19,20], and since maximal activation of the enzyme by NEM requires modification of the SH groups of all three cysteine residues in the homotrimer [20], it would appear that our enzyme preparation consists primarily of enzyme in the reduced, homotrimeric form, rather than as a mixed population of subunits.

3.1. S-Nitrosylation of microsomal GST by NO donors

For the determination of protein *S*-nitrosothiol content, other investigators have used photolytic cleavage of the S–NO bond to release NO from the protein prior to chemiluminescence detection of NO [21,22]. In our study, we chose to remove NO from the protein by transnitrosation of the NO moiety to cysteine. This was followed by the Cu^{2+}-catalyzed decomposition of *S*-nitrosocysteine to release NO, which occurs at a much greater rate than the Cu^{2+}-catalyzed decomposition of protein *S*-nitrosothiols [16]. The NO released was quantitated by redox chemiluminescence. The advantage of this methodology is its simplicity, since all that is required is the addition of the protein of interest to a solution containing Cu^{2+} and excess cysteine, followed by sampling of the headspace gas. The reaction was essentially complete within 30 sec, since no increase in signal was observed when sampling was performed between 30 sec and 10 min. The NO content of the samples was standardized using solutions of GSNO to generate a standard curve (Fig. 2). NO released from GSNO concentrations of 50 nM was quantitated easily. Since the microsomal GST was exposed to an excess of the NO donor, it was necessary to remove the intact NO donor prior to the determination of protein *S*-nitrosothiol content. For GSNO, this was accomplished by repeated washing and concentration using a centrifugal filter device; for DEA/NO, samples were placed on ice for 5–6 hr to allow for the spontaneous release and dissipation of the remaining NO from DEA/NO. The appropriate control samples, in which protein was omitted, were performed in parallel. The NO signal from these samples was less than 2% of the signal obtained in the presence of protein. Incubation of microsomal GST with GSNO or DEA/NO resulted in *S*-nitrosylation of about 45–50% of the protein, when assayed 5–6 hr after the initial incubation with the NO donor (Table 1).

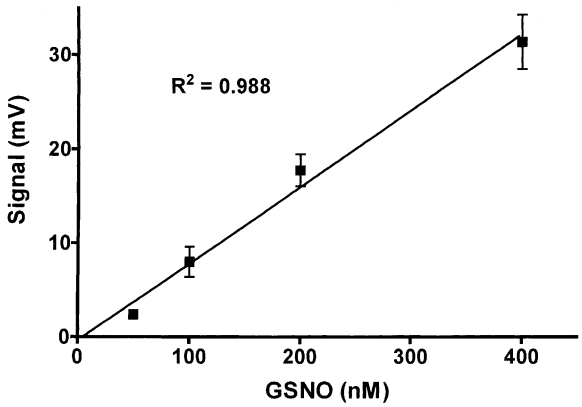

Fig. 2. GSNO standard curve generated using Cu^{2+}-mediated decomposition of GSNO followed by redox chemiluminescence detection of released NO. Data are means ± SD (N = 6).

In parallel with the assessment of *S*-nitrosylation, we measured the free SH content of the enzyme using DTNB.

Table 1
Free sulfhydryl and *S*-nitrosothiol content of microsomal GST after treatment with NO donors

Treatment	Free SH groups (% of control)	Protein *S*-nitrosothiol content (% of control free SH)
Control	100 ± 12 (6)	ND
GSNO	16.3 ± 2.4 (3)	44 ± 9.6 (6)
DEA/NO	12.0 ± 1.4 (3)	48 ± 10 (10)

Free sulfhydryl content of the enzyme was assayed using DTNB, prior to (control) and 10 min after exposure to 1 mM GSNO or 0.5 mM DEA/NO. The procedure for the removal of the NO donors from samples lasted 5–6 hr, after which protein *S*-nitrosothiol content was measured as NO release from the enzyme after transnitrosation of NO to cysteine followed by Cu^{2+}-catalyzed decomposition of *S*-nitrosocysteine, and redox chemiluminescence detection of the released NO. The amount of NO released is expressed as a percentage of the total SH content of the enzyme. Data are means ± SD of the number of determinations shown in parentheses. ND, not detected.

Immediately after the 10-min incubation with NO donors, the free SH content was reduced to about 15% of control (Table 1), indicating that the majority of the Cys49 sulfhydryl had been modified. However, when assessed at the same time that *S*-nitrosylation was assessed (i.e. 5–6 hr later), the free SH content had increased to about 30 and 60% of control for GSNO- and DEA/NO-treated enzyme, respectively, suggesting that denitrosylation of the enzyme had occurred over time.

3.2. Effect of NO donors on GST activity

Incubation of microsomal GST with GSNO increased GST activity in a concentration- and time-dependent manner (Fig. 3), with the maximal increase in activity being about 2-fold. To determine whether modification of Cys49 was the basis for this increase in enzyme activity, we used NEM, an alkylating agent, to monitor the state of sulfhydryl groups in the enzyme. As seen in panels A and B of Fig. 3, preincubation of microsomal GST with GSNO prevented further activation of the enzyme by NEM in a concentration- and time-dependent manner, suggesting that the two reagents interact at the same site in the enzyme. To further examine the effects of *S*-nitrosylation of microsomal GST by NO, we used another NO donor, DEA/NO. As seen in Fig. 4A, GSNO and DEA/NO activated the enzyme to a similar extent, and this increase in enzyme activity was prevented by subsequent treatment of the enzyme with DTT, indicating that the modification of the enzyme by GSNO and DEA/NO was reversible. Furthermore, after incubation of GSNO- or DEA/NO-pretreated microsomal GST with DTT, NEM treatment resulted in enzyme activation to an extent similar to that seen when untreated enzyme was exposed to NEM (Fig. 4B), indicating reduction of Cys49 by DTT to the free sulfhydryl form.

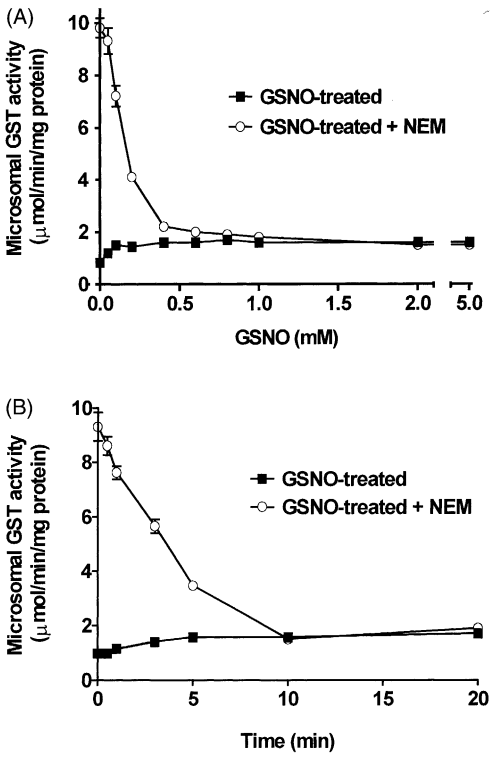

Fig. 3. Effect of GSNO on microsomal GST activity. Purified enzyme (2 μg) was incubated with the indicated concentrations of GSNO at 37° for 10 min (A) or with 1.0 mM GSNO for the indicated times (B). In experiments utilizing NEM, GSNO-treated enzyme was exposed to 1.0 mM NEM for an additional 1 min prior to the assessment of enzyme activity. Data are means ± SD (N = 6).

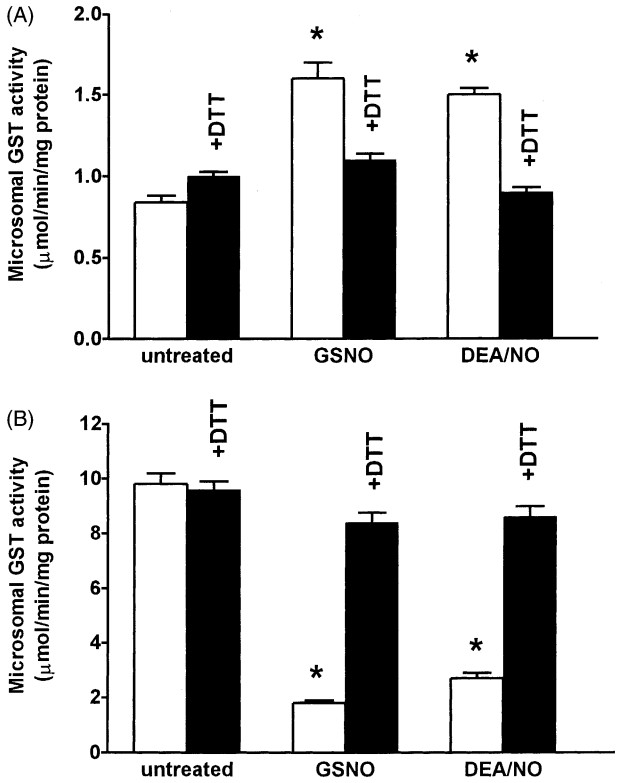

Fig. 4. Effect of sulfhydryl reagents on microsomal GST activity. Purified enzyme (2 μg) was incubated with 1.0 mM GSNO or 0.5 mM DEA/NO at 37° for 10 min and then exposed to 1.0 mM DTT at room temperature for an additional 20 min (A). In panel B, samples were incubated with GSNO or DEA/NO and then incubated with 3.0 mM NEM for 1 min, or were incubated with 1.0 mM DTT prior to treatment with NEM. Data are means ± SD (N = 6). Key: (*) $P < 0.001$, significantly different from untreated (one-way ANOVA, Neuman–Keuls post-hoc test).

In contrast to the results using microsomal GST, treatment of a mixture of rat hepatic cytosolic GSTs with GSNO or DEA/NO resulted in a modest inhibition of GST activity (Fig. 5A) that was reversed by treatment of the enzyme preparation with DTT. Treatment with NEM resulted in a similar degree of inhibition (Fig. 5B), and this degree of inhibition was only slightly greater after pretreatment of the enzyme preparation with GSNO or DEA/NO prior to treatment with NEM (Fig. 5B). We repeated these experiments using purified human GST pi, since the activity of this cytosolic GST isoform is much more sensitive to inhibition after treatment with SH reagents [23,24]. In this case, GST activity was inhibited by about

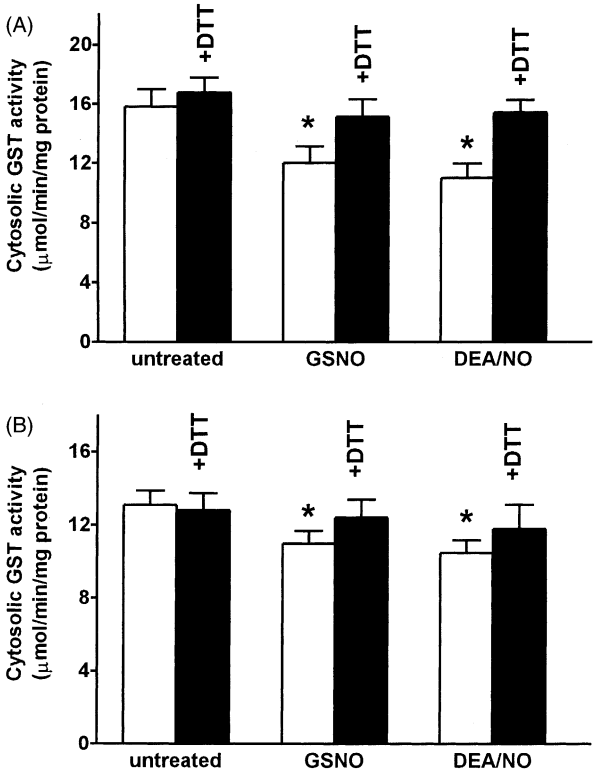

Fig. 5. Effect of sulfhydryl reagents on cytosolic GST activity. Purified enzyme (2 μg) was incubated with 1.0 mM GSNO or 0.5 mM DEA/NO at 37° for 10 min and then exposed to 1.0 mM DTT at room temperature for an additional 20 min (A). In panel B, samples were incubated with GSNO or DEA/NO and then incubated with 3.0 mM NEM for 1 min, or were incubated with 1.0 mM DTT prior to treatment with NEM. Data are means ± SD (N = 6). Key: (*) $P < 0.001$, significantly different from untreated (one-way ANOVA, Neuman–Keuls post-hoc test).

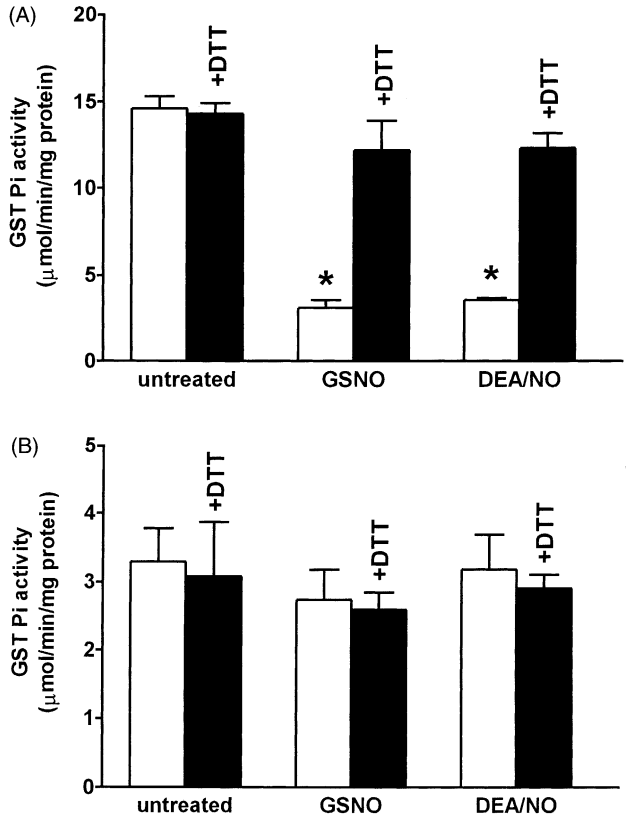

Fig. 6. Effect of sulfhydryl reagents on human GST pi activity. Purified enzyme (2 μg) was incubated with 1.0 mM GSNO or 0.5 mM DEA/NO at 37° for 10 min and then exposed to 1.0 mM DTT at room temperature for an additional 20 min (A). In panel B, samples were incubated with GSNO or DEA/NO and then incubated with 3.0 mM NEM for 1 min, or were incubated with 1.0 mM DTT prior to treatment with NEM. Data are means ± SD (N = 3). Key: (*) $P < 0.001$, significantly different from untreated (one-way ANOVA, Neuman–Keuls post-hoc test).

80% after exposure to GSNO, DEA/NO, or NEM (Fig. 6 A and B). As was observed with the rat liver cytosolic GSTs, the inhibitory effects of GSNO and DEA/NO were reversed by DTT, and the inhibitory effect of NEM was only slightly increased when the enzyme was first treated with GSNO or DEA/NO (Fig. 6B). In a final series of experiments, we used rat hepatic microsomes rather than purified enzyme preparations. In this case, GST activity was unaffected by treatment with GSNO or DEA/NO (Fig. 7A), but was increased about 5-fold by NEM (Fig. 7B). Similar to the results using purified microsomal GST, the NEM-mediated increase in activity was inhibited by prior treatment with NO donors, and this effect was prevented by DTT (Fig. 7B).

4. Discussion

In this study, we examined the effect of NO donors on the activities of cytosolic and microsomal GSTs. To support the contention that alterations in microsomal GST activity after exposure to NO donors were due primarily to *S*-nitrosylation of Cys49 in the enzyme rather than to *S*-glutathiolation (in the case of GSNO) or *S*-oxidation (by either NO donor), we assayed the NO content after incubation of the enzyme with GSNO or DEA/NO. Following the 5- to 6-hr period during which the unreacted NO donor was removed from the samples, we found that about 50% of the SH groups in the enzyme were *S*-nitrosylated, based on the SH content of the enzyme prior to exposure to NO donors, thus indicating the major contribution of *S*-nitrosylation to the effects of GSNO and DEA/NO on enzyme activity. However, immediately after the 10-min incubation of the enzyme with GSNO or DEA/NO (the time at which enzyme activity measurements were performed), the free SH content was reduced by about 85%, leaving the fate of about 35% of the SH content unaccounted for during the period of the sample work-up (Table 1).

In the case of DEA/NO, the ''missing'' SH could only be due to sulfenic acid (S–OH) formation or to denitrosylation of protein *S*-nitrosothiols during the time of the sample work-up. Sulfenic acids are generally very unstable and highly reactive, although proteins with stable S–OH groups have been described [25]. The fact that the SH content of microsomal GST increased from 15 to about 60% during

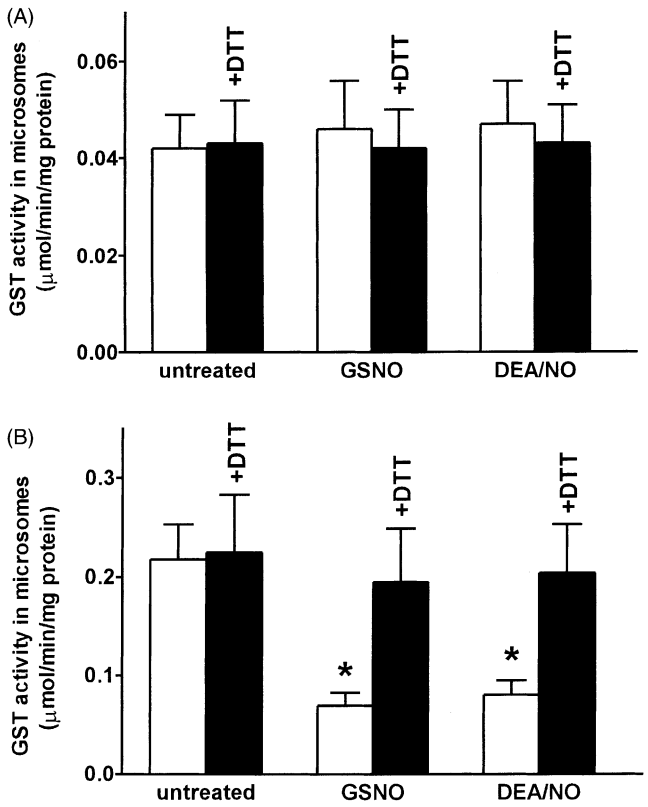

Fig. 7. Effect of sulfhydryl reagents on GST activity in rat hepatic microsomes. Microsomal protein (30–50 μg) was incubated with 1.0 mM GSNO or 0.5 mM DEA/NO at 37° for 10 min and then exposed to 1.0 mM DTT at room temperature for an additional 20 min (A). In panel B, samples were incubated with GSNO or DEA/NO and then incubated with 3.0 mM NEM for 1 min, or were incubated with 1.0 mM DTT prior to treatment with NEM. Data are means ± SD (N = 3). Key: (*) $P < 0.001$, significantly different from untreated (one-way ANOVA, Neuman–Keuls post-hoc test).

the 5- to 6-hr period of the sample work-up would rule out S–OH formation since there is no source of reducing equivalents for the reduction of S–OH back to the free SH form. Further *S*-oxidation of S–OH to sulfinic ($S–O_2H$) or sulfonic ($S–O_3H$) acids during the initial 10-min incubation with DEA/NO can also be ruled out since the effects of NO donors were reversed completely by DTT (DTT does not reduce sulfur in these higher oxidation states). The more likely possibility is denitrosylation of protein *S*-nitrosothiol by spontaneous hydrolysis [26] or by hydrolysis catalyzed by acidic or basic amino acid residues [27]. For GSNO, the additional possibility of *S*-glutathiolation of Cys49 must be considered. After treatment with GSNO, the free SH content of the enzyme was increased to only about 30% after 5–6 hr, leaving the possibility that a portion of the SH modification was due to *S*-glutathiolation. Whether *S*-glutathiolation occurred during the initial 10-min incubation with GSNO, or during the period of the sample work-up is the subject of an ongoing investigation. However, it is generally considered that *S*-nitrosylation is the favoured initial modification after treatment with GSNO, and that protein *S*-nitrosothiols act as intermediates in the formation of protein *S*-glutathiolation reaction products [27].

Regardless of the status of all of the Cys49 SH groups after treatment with different NO donors, the functional consequences of modifying the enzyme with either GSNO or DEA/NO were the same. Treatment of purified microsomal GST with GSNO or DEA/NO resulted in a 2-fold increase in catalytic activity, suggesting that in addition to covalent modification by reagents such as NEM, nitrosylation of the Cys49 can also alter enzyme activity, albeit not to the same magnitude. More significant, perhaps, is that nitrosylation of Cys49 prevented further covalent modification of the enzyme by NEM, so that the large (10- to 15-fold) increase in enzyme activity normally observed after NEM treatment was absent. The inhibition of NEM activation by NO donors occurred in both purified enzyme preparations and hepatic microsomes. However, in contrast to the purified microsomal GST preparation, there was no observed increase in enzyme activity after treatment of hepatic microsomes with NO donors. This probably was due to contamination of the microsomes with cytosolic GSTs, which would contribute to the enzyme activity of the preparation, but would be inhibited by NO donors, thus offsetting any activation of the microsomal GST in the preparation. We used DTT to assess whether modifications of the enzyme after treatment with NO donors were reversible. Reversibility of enzyme modifications by DTT after treatment with NO donors is suggestive of *S*-nitrosylation reactions having occurred [28–31], although this reagent cannot distinguish between *S*-nitrosylation, disulfide formation, or sulfenic acid formation. The inhibitory effects of GSNO and DEA/NO on NEM activation of the enzyme were reversed completely by DTT. Taken together, the data indicate that NO donors and NEM act at a common site in the enzyme, Cys49, to alter enzyme activity. The reversibility of the effects of NO donors by DTT also indicates that oxidation of Cys49 to higher oxidation states of sulfur does not occur.

With respect to the cytosolic GSTs, Clark and Debnam [11] reported that the mode of inhibition by GSNO (0 to 0.8 mM) of rat liver cytosolic GST 1-1 and 1-2 subunits was competitive with respect to GSH and non-competitive with respect to CDNB, and that there was no evidence for covalent modification of the enzymes. It was suggested that GSNO inhibited GST activity by binding to the same site as GSH at the active center of the enzyme [11]. However, we found that DEA/NO inhibited the cytosolic GSTs to the same extent as GSNO, suggesting that NO mediates the inhibitory effect on enzyme activity (Fig. 5A), presumably by *S*-nitrosylation of sulfhydryl groups in the enzyme. The results of experiments utilizing DTT and NEM are consistent with this interpretation. Similar to the findings with microsomal GST, the effect of GSNO and DEA/NO on cytosolic GST activity (inhibition of enzyme activity in this case) was reversed by DTT (Fig. 5A). Previous studies have demonstrated either a negligible or modest inhibitory

effect of NEM on cytosolic GST activity [15,24,32–34]. In the current study, cytosolic GST activity was inhibited by about 17% after treatment with NEM, and this degree of inhibition was increased only slightly by pretreatment of the enzyme preparation with GSNO or DEA/NO prior to treatment with NEM (Fig. 5B). This minor additional component of enzyme inhibition was reversed by DTT. Together, these data suggest that, for the most part, NEM and NO donors act on a common population of sulfhydryl groups to affect catalytic activity. In contrast to the modest inhibitory effects of NEM and NO donors on rat hepatic cytosolic GSTs, these reagents had a marked inhibitory effect on the activity of the human GST pi isoform. However, the effects of these reagents were qualitatively the same in both enzyme preparations with respect to the non-additive effects of NEM and NO donors on the inhibition of enzyme activity, and the reversal of NO donor-mediated inhibition by DTT.

For the most part, enzyme *S*-nitrosylation by NO donors results in inhibition of enzyme activity [21,28–31,35], such as that which occurred after treatment of cytosolic GSTs with GSNO and DEA/NO. In contrast, the microsomal GST is somewhat unique in that *S*-nitrosylation results in increased catalytic activity. Previous findings of increased microsomal GST activity after exposure to oxidants such as hydrogen peroxide and superoxide [36], and that microsomal GST inhibits lipid peroxidation [37], have led to the suggestion that this GST isoform may play a protective role under conditions of oxidative stress. The results of the current study raise the possibility that the microsomal GST may have a protective role under conditions of nitrosative stress as well. Alternatively, prevention by *S*-nitrosylation of the much larger increase in enzyme activity such as occurs after covalent modification of the enzyme (i.e. 2-fold increase in activity with NO vs. 15-fold with NEM) raises the possibility that nitrosative stress could limit the protective effects afforded by activation of the enzyme under conditions of oxidative stress.

Acknowledgments

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