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IN VITRO INHIBITION OF GLUTATHIONE REDUCTASE BY ARSENOTRIGLUTATHIONE

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Abstract—Arsenotriglutathione, a product of the reaction of arsenate or arsenite with glutathione, is a mixed-type inhibitor (K_i = 0.34 mM) of the *in vitro* reduction of glutathione disulfide by purified yeast glutathione reductase. Notably, arsenotriglutathione was a 10-fold more potent inhibitor than either arsenite or glutathione. The inhibition of glutathione reductase by arsenotriglutathione was partly reversed by the addition of *meso*-2,3-dimercaptosuccinic acid (DMSA). However, high concentrations of DMSA also inhibited the reduction of glutathione disulfide by the yeast enzyme (IC_{50} of 7 mM with 0.1 mM glutathione disulfide). Ultrafiltration of the enzyme–arsenotriglutathione complex recovered about 74% of the original (non-inhibited) activity, suggesting that the inhibition of glutathione reductase by arsenotriglutathione had both reversible and irreversible components. The relatively high potency of arsenotriglutathione as an inhibitor of glutathione reductase may alter the reduction of glutathione disulfide and affect the availability of glutathione that is required for the reduction of arsenate to arsenite and for the formation of the arsenotriglutathione complex.

Key words: arsenotriglutathione; glutathione reductase; inhibition; arsenic; glutathione; arsenic toxicity

Because the toxicity of arsenic is affected by valence [1], both the mechanism of reduction of As^V to As^{III} and the relation between the reductive metabolism of arsenic and its subsequent conversion to mono-, di- and trimethylated species have been topics of considerable interest. GSH†, the most abundant low M_r thiol-containing species in cells [2], reduces As^V to As^{III} and forms stable complexes with many trivalent arsenic compounds including inorganic As^{III}, CH_3As^{III}, $(CH_3)_2As^{III}$ and $C_6H_6As^{III}$ [3–5]. $As^{III}(GS)_3$, which readily donates As^{III} to dithiol-containing molecules [6], is found in intact erythrocytes exposed *in vitro* to arsenate or arsenite [7]. In an *in vitro* system, exogenous GSH is required to support the enzymatic methylation of inorganic As^{III} and CH_3As^{III} [8–10]. The dependence of arsenic methylation on the presence of GSH in *in vitro* assay systems may indicate that complexation of arsenicals by GSH is required for these methylation reactions or that GSH plays a role in the maintenance of the structure and function of the methylating enzymes.

Like arsenicals, selenite has been shown to react with GSH to form a selenodiglutathione complex, $Se(GS)_2$ [11, 12]. This complex is metabolized by glutathione reductase (NADPH:oxidized glutathione oxidoreductase, EC 1.6.4.2) to yield GSSeH and GSH. The formation of $Se(GS)_2$ and its reduction by glutathione reductase are critical steps in the metabolism and disposition of inorganic selenium compounds [13]. Given the similarity between $Se(GS)_2$ and $As^{III}(GS)_3$, it was hypothesized that $As^{III}(GS)_3$ might be a substrate for glutathione reductase, yielding a structural analog of GSSeH, possibly $AsH(GS)_2$. Although $As^{III}(GS)_3$ was found not be a substrate for glutathione reductase, it was found to be a mixed-type inhibitor of the reduction of GSSG by the purified yeast enzyme.

MATERIALS AND METHODS

Reagents. Purified glutathione reductase (Type IV prepared from Baker's yeast), BSA, sodium arsenate, sodium arsenite, GSH, GSSG, NADPH, and DMSA were purchased from Sigma (St. Louis, MO). Sodium [^{73}As]arsenate was obtained from Los Alamos Meson Production Facility (Los Alamos, NM) and [^{35}S]-GSH from NEN (DuPont, Wilmington, DE). [^{73}As]-Arsenite was prepared from [^{73}As]arsenate by the method of Reay and Asher [14]. $As^{III}(GS)_3$ was prepared by reaction of sodium arsenite with GSH at a 1:3 molar ratio. Radiolabeled $As^{III}(GS)_3$ was prepared by reaction of sodium [^{73}As]arsenate with GSH or sodium arsenate with [^{35}S]GSH at a 1:5 molar ratio. Preparation of $As^{III}(GS)_3$ followed procedures developed in this laboratory, and its identity was verified by NMR spectroscopy [5].

Erythrocyte lysate. Rabbit erythrocyte lysate was used as the source of mammalian glutathione reductase. Blood was collected in heparin-containing vacutainers (Becton Dickinson, Rutherford, NJ) by

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† Abbreviations: GSH, glutathione; CH_3As^{III}, monomethylarsenic; $(CH_3)_2As^{III}$, dimethylarsenic; $C_6H_6As^{III}$, phenylarsenic; $As^{III}(GS)_3$, arsenotriglutathione; $Se(GS)_2$, selenodiglutathione; GSSeH, selenopersulfide; GSSG, glutathione disulfide; and DMSA, *meso*-2,3-dimercaptosuccinic acid.

venipuncture of the marginal ear veins of adult female New Zealand White rabbits (Hazelton, Denver, PA). Blood was centrifuged at 3000 *g* for 15 min at 4°. After removal of plasma, cells were washed twice with modified Krebs–Ringer buffer with subsequent centrifugation at 3000 *g* for 5 min at 4°. Washed cells were lysed with ice-cold distilled deionized water. Erythrocyte membranes were removed by centrifugation at 29,000 *g* for 15 min at 4°. The concentration of hemoglobin in lysate, as determined using a Total Hemoglobin diagnostic kit (Sigma), was 62 mg/mL.

Glutathione reductase assay. The glutathione reductase assay monitored the oxidation of NADPH consumed in the reduction of GSSG by the change in 340 nm absorbance. The assay was performed at 37° in a 1-cm spectrophotometric cuvette in 0.15 M phosphate buffer that contained 6 mM EDTA (pH 7) with 0.23 mM NADPH and 0.3 μg of yeast glutathione reductase or 30 μL of rabbit erythrocyte lysate; 0.01 to 10 mM GSSG was used in the assay. To investigate the effect of inhibitors, assays were performed at a constant GSSG concentration (0.1 mM), and inhibitors [$As^{III}(GS)_3$, GSH, and arsenite] were added at final concentrations ranging from 0.02 to 40 mM. The final volume of the assay mixture was 2.6 mL. Reactions were started by addition of NADPH, and 340 nm absorbance was measured at 20-sec intervals for 3 min.

Analysis of [^{73}As]$As^{III}(GS)_3$ *and* $As^{III}([^{35}S]GS)_3$ *in the assay mixture.* Here, the enzyme assay was performed at 1 mM GSSG and in the presence of radiolabeled [^{73}As]$As^{III}(GS)_3$ or $As^{III}([^{35}S]GS)_3$ (4.8 mM). After a 3-min incubation period, the integrity of the complex in the assay mixture was determined by TLC on SigmaCell type 100 cellulose plates (Sigma) developed with an isopropanol : acetic acid : water (10 : 1 : 5) solvent system.* Standards included radiolabeled $As^{III}(GS)_3$, arsenate, arsenite, GSH, and unlabeled GSSG prepared in the assay mixture, which contained enzyme, NADPH and GSSG in 0.15 M phosphate buffer with 6 mM EDTA (pH 7). Developed TLC plates were sprayed with ninhydrin to visualize amino acid residues. Radioactive compounds were detected using an AMBIS 4000 imaging detector (Ambis, San Diego, CA).

Reversibility of in vitro *inhibition.* To test the reversibility of inhibition by $As^{III}(GS)_3$, glutathione reductase (0.6 μg) was dissolved in 76 μL of 0.15 M phosphate buffer with 6 mM EDTA (pH 7) and 20 mg of BSA/mL. BSA was added to reduce the nonspecific binding of glutathione reductase to ultrafiltration membranes. This mixture was incubated for 5 min at 37° in the presence of 27.5 μmol of [^{73}As]$As^{III}(GS)_3$. A control mixture that contained only enzyme and BSA in 0.15 M phosphate buffer with 6 mM EDTA (pH 7) was processed in parallel. Mixtures were ultrafiltered with Microcon microconcentrators (Amicon, Beverly, MA) with a 10-kDa cutoff by centrifugation at 12,000 *g* for 35 min at 4°. Retentates were washed twice with 50 μL of ice-cold distilled deionized water with centrifugation for 15 min at 4°. Retentates were resuspended in 50 μL of ice-cold water. The original incubation mixtures and the resuspended retentates from $As^{III}(GS)_3$-treated and control samples were analyzed for glutathione reductase activity, using 1 mM GSSG as the substrate. The final concentration of $As^{III}(GS)_3$ in the assay mixture was 10 mM. [^{73}As] in ultrafiltrates and retentates was measured with a Minaxi γ 5000 counter (Packard).

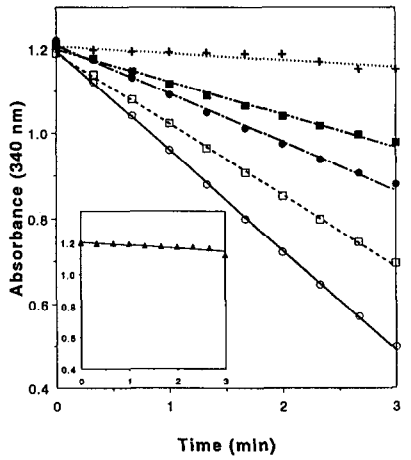

Fig. 1. Oxidation of NADPH by yeast glutathione reductase ([GSSG] = 1 mM) in the presence or absence of $As^{III}(GS)_3$: 0 mM (○), 1.5 mM (□), 3.8 mM (●), and 7.7 mM (■). Nonenzymatic oxidation of NADPH in the absence of $As^{III}(GS)_3$ is also shown (+). Inset: oxidation of NADPH by yeast glutathione reductase (no added GSSG) in the presence of 1 mM $As^{III}(GS)_3$ (▲).

RESULTS

$As^{III}(GS)_3$ as a substrate of glutathione reductase. No significant enzymatic activity was found when $As^{III}(GS)_3$ was tested as a possible substrate for the purified yeast enzyme at a constant concentration of NADPH and in the absence of GSSG (Fig. 1, inset). Over a concentration range of 0.02 to 20 mM $As^{III}(GS)_3$, oxidation of NADPH did not exceed that produced in an assay mixture that contained all components except enzyme during a 3-min incubation at 37°.

In vitro *inhibition of glutathione reductase by $As^{III}(GS)_3$.* In an assay mixture that contained 1.0 mM GSSG, $As^{III}(GS)_3$ inhibited the enzymatic oxidation of NADPH by glutathione reductase (Fig. 1). However, up to 30 mM $As^{III}(GS)_3$ did not affect the nonenzymatic oxidation of NADPH. At higher $As^{III}(GS)_3$ concentrations, nonenzymatic oxidation of NADPH increased markedly (data not shown). A 50% reduction in glutathione reductase activity (IC_{50}) was observed at 3.8 mM $As^{III}(GS)_3$. When the assay was run with 0.1 mM GSSG, the IC_{50} was 1.5 mM. Maximal inhibition (85–90%) occurred when the $As^{III}(GS)_3$ concentration reached 6 and 30 mM for GSSG concentrations of 0.1 and 1.0 mM, respectively. A higher concentration of $As^{III}(GS)_3$ in the assay mixture did not inhibit completely the

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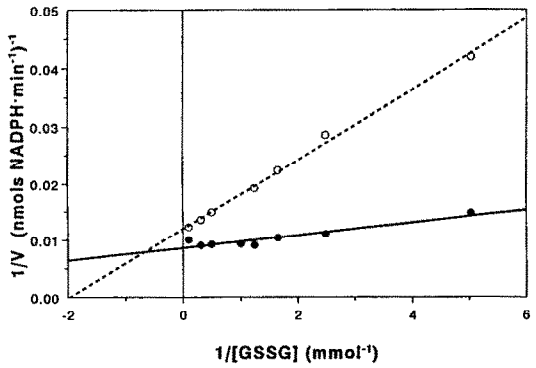

Fig. 2. Double-reciprocal plot ($1/v$ versus 1/[GSSG]) for the activity of yeast glutathione reductase in the presence of 3.8 mM $As^{III}(GS)_3$ (○) and in the absence of inhibitor (●).

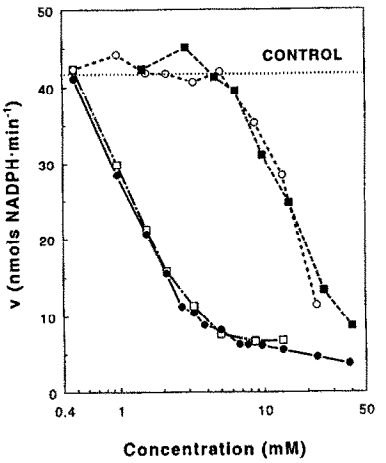

Fig. 4. Inhibition of yeast glutathione reductase by $As^{III}(GS)_3$ (●), arsenite (○), GSH (■), and arsenite and GSH added together at a 1:3 molar ratio (□). [GSSG] = 0.1 mM. Control indicates the activity of the enzyme with no inhibitors present.

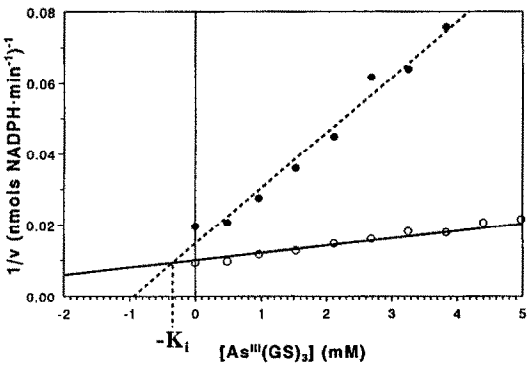

Fig. 3. Dixon plot for the inhibition of the activity of yeast glutathione reductase by $As^{III}(GS)_3$ at 0.1 mM GSSG (●) and 1.0 mM GSSG (○).

reductive activity of the enzyme, despite a 2-min preincubation of $As^{III}(GS)_3$ and the enzyme before addition of GSSG (data not shown).

The double-reciprocal plot, $1/v$ versus 1/[GSSG], showed that 3.8 mM $As^{III}(GS)_3$ was a mixed-type inhibitor, increasing the K_m (0.11 mM) and decreasing the V_{max} (111 nmol NADPH/min) for the enzymatic reduction of GSSG (Fig. 2). A Dixon plot, $1/v$ versus [I] at different concentrations of substrate [15], estimated the K_i as ≈0.34 mM $As^{III}(GS)_3$ (Fig. 3)

Comparison of inhibitory effects of $As^{III}(GS)_3$, arsenite, and GSH. $As^{III}(GS)_3$ was approximately 10-fold more potent than either arsenite or GSH as an inhibitor of the reduction of 0.1 mM GSSG by glutathione reductase (Fig. 4). The approximate IC_{50} for $As^{III}(GS)_3$ was less than 2 mM; for either arsenite or GSH, it was about 20 mM. Notably, when arsenite and GSH were added directly to the assay mixture at a 1:3 molar ratio, the inhibition of GSSG reduction was equal to that observed after addition of authentic $As^{III}(GS)_3$. These data suggest that the complex could be formed rapidly from its constituents in the assay mixture.

Stability of $As^{III}(GS)_3$ in the in vitro *assay mixture.* Stability of $As^{III}(GS)_3$ during a 3-min incubation in the assay mixture at 37° was examined by TLC using radiolabeled $As^{III}(GS)_3$. No free [^{73}As]arsenite was detected in an assay mixture incubated with [^{73}As]-$As^{III}(GS)_3$ (Fig. 5A, lane 1). The only ^{73}As-labeled compound detected has an R_f value similar to that of authentic [^{73}As]$As^{III}(GS)_3$ (lane 2) and unlike the R_f values for [^{73}As]arsenite (lane 3) and [^{73}As]-arsenate (lane 4). Similarly, $As^{III}([^{35}S]GS)_3$ was a major radiolabeled compound (> 90% of total ^{35}S) found in an assay mixture incubated with $As^{III}([^{35}S]$-$GS)_3$ (Fig. 5B, lane 5). Although not visible in the radiograph, about 6% of the total ^{35}S was detected in the position of free [^{35}S]GSH. A similar distribution of [^{35}S] was found also for an $As^{III}([^{35}S]GS)_3$ standard (lane 6) prepared in the assay mixture. Part of the [^{35}S]GSH carrier-free standard (lane 7) was found to be oxidized to [^{35}S]GSSG.

Effect of DMSA on in vitro *inhibition of glutathione reductase by $As^{III}(GS)_3$.* The effect of DMSA on the activity of glutathione reductase in the presence of 3.8 mM $As^{III}(GS)_3$ or 26 mM GSH was examined (Fig. 6). DMSA was a potent inhibitor of the enzyme (IC_{50} of ≈7 mM at 0.1 mM GSSG). Lower concentrations of DMSA (up to 5 mM) partly reversed the inhibition by $As^{III}(GS)_3$; however, at higher DMSA concentrations, its inhibitory effect predominated. DMSA did not reverse the inhibition of glutathione reductase by GSH.

Reversibility of in vitro *inhibition of glutathione reductase by $As^{III}(GS)_3$.* The reversibility of the inhibition of glutathione reductase by $As^{III}(GS)_3$ was examined using ultrafiltration of the enzyme treated with [^{73}As]$As^{III}(GS)_3$ in the presence of BSA. The reductase activity of $As^{III}(GS)_3$-treated or control enzyme before and after ultrafiltration was deter-

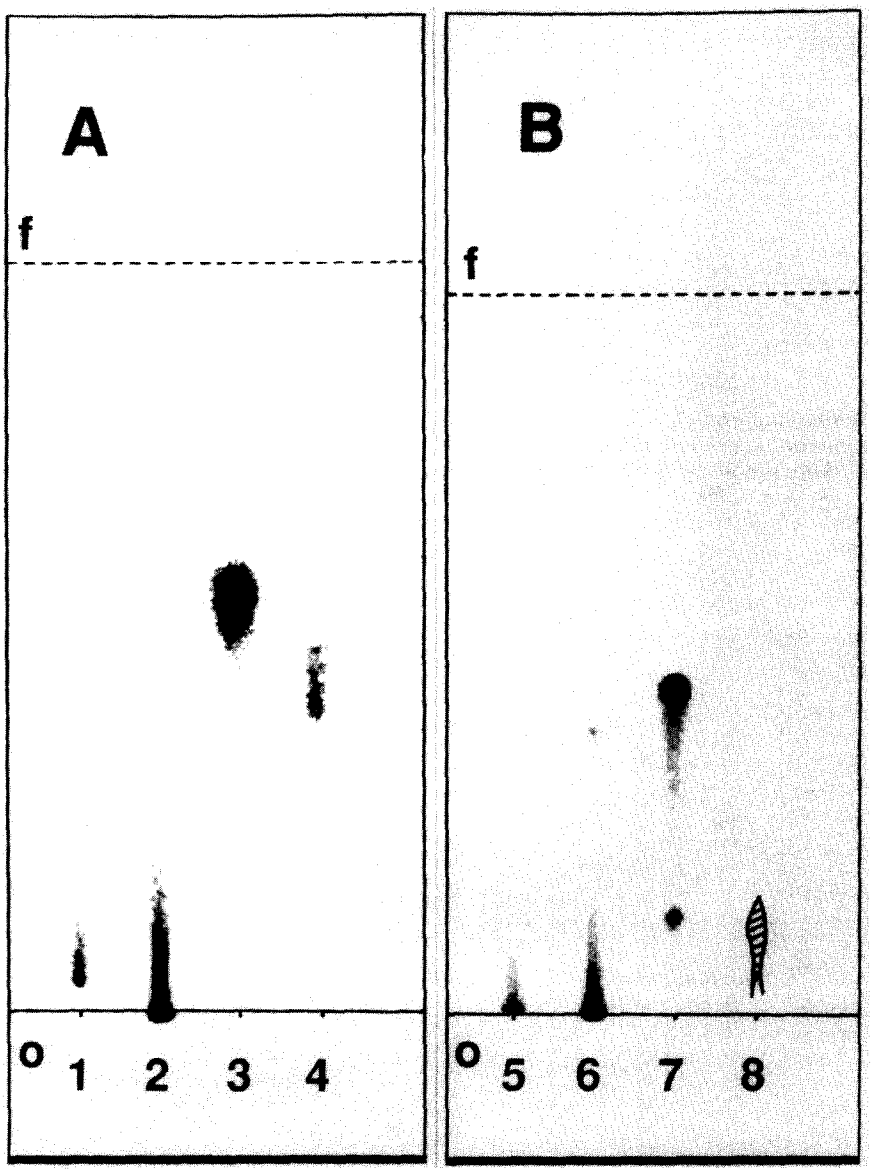

Fig. 5. Thin-layer chromatograms of assay mixtures incubated with [^{73}As]$As^{III}(GS)_3$ (panel A) or $As^{III}([^{35}S]GS)_3$ (panel B) and authentic standards. Lane 1, assay mixture incubated with [^{73}As]$As^{III}(GS)_3$; lane 2, [^{73}As]$As^{III}(GS)_3$ standard; lane 3, [^{73}As]arsenite standard; lane 4, [^{73}As]arsenate standard; lane 5, assay mixture incubated with $As^{III}([^{35}S]GS)_3$; lane 6, $As^{III}([^{35}S]GS)_3$ standard; lane 7, [^{35}S]GSH standard; and lane 8, GSSG standard (ninhydrin stain, ▨). Front (f) and origin (o) are labeled.

mined (Table 1). Ultrafiltration of the enzyme–inhibitor complex recovered 74.1% of the original enzymatic activity (81.3% of the activity when compared with the activity of uninhibited enzyme after ultrafiltration). After ultrafiltration and two washes, about 16% of ^{73}As from radiolabeled $As^{III}(GS)_3$ was found in the retentate that contained enzyme and BSA.

In vitro *inhibition of glutathione reductase activity in rabbit erythrocyte lysate by* $As^{III}(GS)_3$. The inhibitory effect of $As^{III}(GS)_3$ on glutathione reductase activity in rabbit erythrocyte lysate was examined using an assay mixture containing either 0.27 or 1.0 mM GSSG. $As^{III}(GS)_3$ inhibited the enzymatic reduction of GSSG with an estimated IC_{50} of 2.5 mM at 1 mM GSSG; for 0.27 mM GSSG, the estimated IC_{50} was 1.0 mM. GSH and arsenite were less potent inhibitors with an estimated IC_{50} of 9.8 mM for GSH and an estimated IC_{50} of 23.0 mM for arsenite at 0.27 mM GSSG.

DISCUSSION

In 1971, Ganther [11] reported that $Se(GS)_2$, the selenotrisulfide derivative of GSH, was reduced to its persulfide analog (GSSeH) by yeast glutathione reductase (Equation 1). Because glutathione reductase stimulated production of H_2Se from

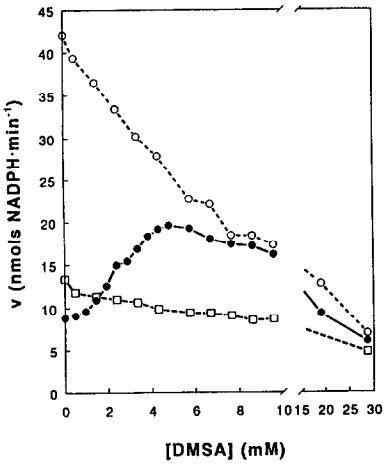

Fig. 6. Effect of DMSA on the inhibition of yeast glutathione reductase by $As^{III}(GS)_3$ and GSH: 3.8 mM $As^{III}(GS)_3$ (●), 26 mM GSH (□), and DMSA alone (○).

Table 1. Activity of uninhibited and inhibited glutathione reductase before and after ultrafiltration*

	Glutathione reductase activity (nmol NADPH oxidized·min^{-1})	
	Prefiltration	Postfiltration
Control	122.63 ± 1.40 (100)†	111.63 ± 4.66 (91.2)
+ $As^{III}(GS)_3$	44.83 ± 3.21 (36.6)	90.93 ± 6.91 (74.1)

* Glutathione reductase activity was assayed in the presence of 1 mM GSSG. The final $As^{III}(GS)_3$ concentration in the inhibited assay system was 10 mM. Values are means ± SD, N = 4.

† Percent activity of control before ultrafiltration.

$Se(GS)_2$, it was proposed [12] that this enzyme was also involved in the second step (Equation 2) of the reduction of $Se(GS)_2$, yielding GSH and H_2Se:

$$Se(GS)_2 + NADPH + H^+ \rightarrow GSSeH + GSH + NADP^+ \quad (1)$$

$$GSSeH + NADPH + H^+ \rightarrow H_2Se + GSH + NADP^+ \quad (2)$$

The *in vitro* uptake of selenite by intact rat erythrocytes was found to be proportional to cellular GSH concentration [13]. Inhibition of rat erythrocyte glutathione reductase by chromate (IC_{50} of 0.35 mM) decreased selenium release from cells, suggesting that release of selenium was dependent upon a reaction catalyzed by glutathione reductase, possibly the reduction of $Se(GS)_2$ [13]. The formation of $Se(GS)_2$ and its subsequent reduction to H_2Se by glutathione reductase are followed by the enzymatic synthesis of mono-, di- and trimethyl selenium compounds [16].

In the work reported here, the possible role of glutathione reductase in the metabolism of $As^{III}(GS)_3$ was examined. Although not a substrate for glutathione reductase, $As^{III}(GS)_3$ was found to be a potent inhibitor of the reduction of GSSG by this enzyme. Kinetic analysis indicated that $As^{III}(GS)_3$ was a linear mixed-type inhibitor (K_i of 0.34 mM). A Dixon plot of the data indicated that the predominant mechanism of action of $As^{III}(GS)_3$ might be competitive inhibition of GSSG reduction [15].

Both GSH and arsenite were found to be at least 10-fold less potent than $As^{III}(GS)_3$ as inhibitors of glutathione reductase, suggesting that $As^{III}(GS)_3$ rather than its constituents is the inhibitory species. In the present study, glutathione reductase was less sensitive to the inhibitory effect of GSH than was reported previously [17]. However, this difference in sensitivity to GSH could be related to differences in assay conditions (e.g. temperature and buffer composition) used in various studies.

In 1962, Mize and Langdon [18] reported purified rat liver glutathione reductase to be extremely sensitive to arsenite (80% inhibition with 0.3 μM arsenite). However, those investigators suggested that an unidentified heavy metal contaminant, not arsenite, inhibited the enzyme. Addition of 0.1 mM sodium arsenite to an assay mixture containing rat liver cytosol completely abolished the activity of a nonspecific nucleotide-dependent disulfide reductase but does not alter glutathione reductase activity [19, 20]. Addition of 1 mM sodium arsenite to an assay mixture containing purified yeast glutathione reductase has no effect on the rate of NADPH oxidation [11]. Other studies with purified yeast glutathione reductase found that addition of BSA to the assay mixture reduces the inhibitory potency of sodium arsenite [12].

The mechanism of inhibition of yeast glutathione reductase by arsenic is thought to involve the oxidation of thiol groups in vicinal cysteine residues in the active site of the enzyme [21]. Kinetic analysis of the inhibition by phenylarsonous (As^{III}) acid is consistent with the formation of a thiol–As bond at the active site of the enzyme. Kinetic analysis of the inhibition of glutathione reductase by *p*-arsanilic (As^{V}) acid is consistent with an oxidation–reduction scheme in which production of As^{III} requires the oxidation of thiol groups of vicinal cysteine residues of the active site. Because arsenic in $As^{III}(GS)_3$ is formally in the trivalent state, its interaction with thiols of vicinal cysteine residues of the active site of glutathione reductase would be expected to resemble that of phenylarsonous (As^{III}) acid. The contribution of the peptide component of $As^{III}(GS)_3$ to the enhanced interaction with the enzyme may be due to interactions between the GSH residues of $As^{III}(GS)_3$ and the GSSG binding site of the active site of the enzyme [22]. A similar oxidation–reduction cycle for arsenic is invoked in a current model for the methylation of this metalloid [23], and the analogy between the interaction of $As^{III}(GS)_3$ with the active site of glutathione reductase and the

interaction of substrate with the active site of putative arsenic methyltransferase(s) warrants further study.

In the present study, DMSA, which is an effective antidote for the treatment of arsenic poisoning [24], partly reversed the inhibition of glutathione reductase by $As^{III}(GS)_3$. However, high concentrations of DMSA also inhibited GSSG reduction by glutathione reductase. The reversal of $As^{III}(GS)_3$-dependent inhibition by DMSA is consistent with the reversal of Cu^{II}-dependent inhibition of glutathione reductase by the addition of mono- and dithiol-containing species [25]. Because DMSA decomposes $As^{III}(GS)_3$ with the donation of As^{III} to vicinal thiols of DMSA [6], the reversal of $As^{III}(GS)_3$-dependent inhibition probably involves the destruction of the complex in the assay system. Partial reversal of $As^{III}(GS)_3$-dependent inhibition of glutathione reductase was also observed in preliminary studies with dithiothreitol (DTT, *threo*-2,3-dihydroxy-1,4-dithiolbutane). Like DMSA, high concentrations of DTT (> 2 mM) also inhibited GSSG reduction by glutathione reductase.

The reversibility of the effect of $As^{III}(GS)_3$ on the activity of purified glutathione reductase was examined in a series of experiments in which ultrafiltration was used to separate the enzyme from the aqueous phase of the assay system. Before ultrafiltration, addition of 10 mM $As^{III}(GS)_3$ to the assay system reduced the activity to about 37% of that found in an uninhibited assay. Inhibition of activity by $As^{III}(GS)_3$ was partly reversed by ultrafiltration in a sample that contained glutathione reductase, BSA and 10 mM $As^{III}(GS)_3$. Here, the retentate contained about 74% of the activity found in an uninhibited assay. Ultrafiltration of a sample that contained only glutathione reductase and BSA yielded a retentate with about 91% of its prefiltration activity. Thus, ultrafiltration does not materially affect the activity of glutathione reductase in this assay system. The activity restored upon ultrafiltration of the assay mixture is likely to be the reversible component of the inhibitory effect of $As^{III}(GS)_3$. The residual inhibition in the ultrafiltered sample is likely due to irreversible interactions between $As^{III}(GS)_3$ and the enzyme. The irreversible component of the interaction between $As^{III}(GS)_3$ and enzyme could involve the donation of As^{III} from the complex to the vicinal thiol residues at the active site of the enzyme. $As^{III}(GS)_3$ also inhibited glutathione reductase activity in rabbit erythrocyte lysate, suggesting that the production of $As^{III}(GS)_3$ in intact erythrocytes [7] could lead to inhibition of glutathione reductase. Inhibition of this enzyme by $As^{III}(GS)_3$ may alter regeneration of GSH from GSSG, diminishing the amount of GSH available to reduce arsenate and to complex arsenite. Limited availability of GSH may significantly affect the reductive metabolism of arsenic and, consequently, its toxic effect. Reduced availability of GSH due to inhibition of glutathione reductase by $As^{III}(GS)_3$ could also affect the systemic toxicity of arsenic. Reduced GSH availability has been reported to increase the cytotoxicity of arsenic *in vivo* and *in vitro* [26–29], and exogenous GSH increases arsenic methyltransferase activity *in vitro* [8–10]. The *in vitro* inhibition of glutathione reductase by $As^{III}(GS)_3$ is the first example of a biological effect of this complex. Additional studies of the effects of this complex in intact organisms are required.

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