Human placenta thioredoxin reductase is a pyridine nucleotide-disulfide oxidoreductase closely related to glutathione reductase but differing from the latter in having a Cys-SeCys (selencysteine) sequence as an additional redox center. Because selenoproteins cannot be expressed yet in heterologous systems, we optimized the purification of the protein from placenta with respect to final yield (1–2 mg from one placenta), specific activity (42 units/mg), and selenium content (0.94 ± 0.03 mol/mol subunit). The steady state kinetics showed that the enzyme operates by a ping-pong mechanism; the value of $k_{cat}$ was 3330 ± 882 min$^{-1}$, and the $K_m$ values were 18 μM for NADPH and 25 μM for *Escherichia coli* thioredoxin. The activation energy of the reaction was found to be 53.2 kJ/mol, which allows comparisons of the steady state data with previous pre-steady state measurements. In its physiological, NADPH-reduced form, the enzyme is strongly inhibited by organic gold compounds that are widely used in the treatment of rheumatoid arthritis; for auranofin, the $K_i$ was 4 nm when measured in the presence of 50 μM thioredoxin. At 1000-fold higher concentrations, that is at micromolar levels, the drugs also inhibited human glutathione reductase and the selencynzyme glutathione peroxidase.

**Human thioredoxin reductase (NADPH + H$^+$ + thioredoxin$\rightarrow$NADP$^+$ + thioredoxin(SH)$_2$) is a homodimeric flavoenzyme with a subunit size of 55.2 kDa (1–6). This enzyme and other mammalian thioredoxin reductases have recently been shown to be selencenzymes (2, 7–10). At present, only two other mammalian thioredoxin reductases have recently been shown in *S.2**ish Anti-Lewisite (2,3-dithiopropanol); DTNB, 5,5′-dithiobis-(2-nitrobenzoate); EBV, Epstein-Barr virus; E$_{50}$, oxidized thioredoxin reductase containing an active site disulfide; GR, glutathione reductase; SeCys, selencysteine; TE, 50 mM Tris-HCl, 1 mM EDTA adjusted to pH 7.6 at 25 °C; TrxS$_2$, *E. coli* thioredoxin in oxidized form.

Human placenta thioredoxin reductase was isolated and characterized. The enzyme has a subunit size of 55.2 kDa and a $K_m$ value of 18 μM for NADPH. The activation energy of the reaction was found to be 53.2 kJ/mol. The enzyme is strongly inhibited by organic gold compounds, which are widely used in the treatment of rheumatoid arthritis. For auranofin, the $K_i$ was 4 nm when measured in the presence of 50 μM thioredoxin. At 1000-fold higher concentrations, the drugs also inhibited human glutathione reductase and the selencynzyme glutathione peroxidase.

**Materials**

Frozen placentas were kindly provided by Dr. J. Wacker (Department of Obstetrics and Gynecology, Heidelberg University). Purification of human thioredoxin reductase from placenta is delineated below. Recombinant *Escherichia coli* TrxS$_2$ with an $E_{50}$ of 13.7 nm$^{-1}$ cm$^{-1}$ (17, 18) and human glutathione reductase with an $E_{50}$ of 11.3 nm$^{-1}$ cm$^{-1}$ (19) were produced and isolated as described. Human glutathione peroxidase was purchased from Sigma.

Auranofin was obtained from ICN, and aurothioglucose, thioglucose, gold(III)chloride, and British Anti-Lewisite (BAL) (2,3-dithiopropanol)
were from Sigma. Precast gels (12% polyacrylamide) and the protein dye assay were from Bio-Rad, and molecular weight standards were from Amersham Pharmacia Biotech. All reagents were of the highest available purity.

**Enzyme Assays**

All assays were conducted at 25 °C in a total assay volume of 1 ml. 

**Thioredoxin Reductase Activity**—For the purification procedure and the inhibition studies, the DTNB reduction assay (4) proved to be sufficiently specific. The enzyme was added to an assay mixture of 100 mM potassium phosphate, 2 mM EDTA, pH 7.4, and 3 mM DTNB (using a 100 mM stock solution in Me$_2$SO); after initiating the reaction with the addition of NADPH (200 $\mu$M final concentration), the increase in absorbance at 412 nm was monitored. 1 enzyme unit is defined as the NADPH-dependent production of 2 $\mu$mol of 2-nitro-5-thiobenzoate ($\varepsilon$$_{340}$ nm = 13.6 $\text{mM}^{-1} \cdot \text{cm}^{-1}$) per min.

In steady state kinetic studies, the assay mixture contained 100 mM potassium phosphate, 2 mM EDTA, pH 7.4, and five different concentrations both of Trx$_2$ (range 5–45 $\mu$M) and of NADPH (range 5–100 $\mu$M; $\varepsilon$$_{340}$ nm = 6.22 $\text{mM}^{-1} \cdot \text{cm}^{-1}$). The reaction was started with thioredoxin reductase (final concentration 4 $\mu$M TrxR subunits), and the decrease in absorbance at 340 nm was determined during the linear phase. 1 enzyme unit is defined as the conversion of 1 $\mu$mol of NADPH per min. Each combination of [NADPH] and [Trx] was repeated six times, and the mean values were used for computing the kinetic constants.

**Glutathione Reductase Activity**—Glutathione reductase activity was measured in an assay mixture consisting of 47 mM potassium phosphate, 1 mM EDTA, 200 mM KCl, pH 6.9, and 100 $\mu$M NADPH; after the addition of glutathione disulfide (1 mM final concentration), the consumption of NADPH was monitored as the decrease in absorbance at 340 nm.

**Glutathione Peroxidase Activity**—Glutathione peroxidase activity was determined in a GR-coupled assay according to Beutler (20). The assay mixture (100 mM Tris-HCl, 1 mM EDTA, pH 8.0, 4 units/ml glutathione peroxidase, 200 mM reduced glutathione, 100 $\mu$M NADPH, and glutathione peroxidase) was equilibrated for 10 min; then the substrate t-butyhydroperoxide (1 mM final concentration) was added and the consumption of NADPH was monitored. We increased the activity of glutathione reductase in the assay from 1 unit/ml (20) to 4 units/ml to assure that this ancillary enzyme was not rate-limiting in the presence of organic gold compounds.

**Protein Assay**—Protein was determined using the Bio-Rad dye assay with bovine serum albumin as a standard.

**Thioredoxin Reductase Purification**

Because of the potential risk of infection, laboratory biosafety regulations (3, 21) were strictly obeyed in the first steps including acetone precipitation. Unless otherwise stated, all procedures were carried out at 4 °C. The TE buffer used throughout the preparation consisted of 50 mM Tris-HCl, 1 mM EDTA, pH 7.6.

**Chloroform-1-butanol Extraction**—(22) A frozen placenta of approximately 500 g was cut with a stainless steel saw into slices (about 1 $\times$ 3 $\times$ 10 cm). The slices were cleaned mechanically from debris with a cover slide, weighed out, and transferred to plastic bags. Per 1 ml of chloroform-butanol extract, 0.85 ml samples of TrxR (700 nM subunits) containing 200 $\mu$M FAD, 40 $\mu$M phenylmethylsulfonyl fluoride in TE buffer (and 100 $\mu$M NADP$^+$; after the addition of thioredoxin reductase activity, and SDS-polyacrylamide gel electrophoresis analysis) homogenized (more than 95% pure) thioredoxin reductase.

**Sephadex G-200 Gel Filtration**—To remove trace impurities, the above fraction may be applied to a Sephadex G-200 column (Amersham Pharmacia Biotech) in a jacketed chromatography tube. The tube was cooled to 6 ± 1 °C, the exact temperature being crucial for the purification success. The column was consecutively washed with 60 ml of TE buffer, 30 ml of 100 mM KCl in TE buffer, 20 ml of 200 mM KCl in TE, 30 ml of 100 mM KCl in TE, 60 ml of 2-fold diluted TE, 60 ml of 500 $\mu$M NADH in TE, 60 ml of TE, 60 ml of 100 $\mu$M NADP$^+$ in TE, and 30 ml of 300 $\mu$M NADP$^+$ in TE. Finally, TrxR activity was eluted with 750 $\mu$M NADP$^+$ in TE buffer, concentrated, and washed with 60 ml of TE, which had been equilibrated with TE buffer, and the pH was adjusted to 7.6 using 100 mM HCl. This fraction was called the DEAE-cellulose eluate.

2.5'-ADP-Sepharose 4B Affinity Chromatography—The above fraction was applied to a 30-ml (1.5 $\times$ 17 cm) 2.5'-ADP-Sepharose 4B column (Amersham Pharmacia Biotech) in a jacketed chromatography tube. The tube was cooled to 6 ± 1 °C, the exact temperature being crucial for the purification success. The column was consecutively washed with 60 ml of TE buffer, 30 ml of 100 mM KCl in TE buffer, 20 ml of 200 mM KCl in TE, 30 ml of 100 mM KCl in TE, 60 ml of 2-fold diluted TE, 60 ml of 500 $\mu$M NADH in TE, 60 ml of TE, 60 ml of 100 $\mu$M NADP$^+$ in TE, and 30 ml of 300 $\mu$M NADP$^+$ in TE. Finally, TrxR activity was eluted with 750 $\mu$M NADP$^+$ in TE buffer, concentrated, and washed with 60 ml of TE buffer in a Centricons 30. This solution, the 2.5'-ADP-Sepharose eluate, contained (on the basis of absorption spectra (7), specific activity, and SDS-polyacrylamide gel electrophoresis analysis) homogeneous (more than 95% pure) thioredoxin reductase.

**RESULTS**

Enzyme Purification—Because it is not yet possible to produce recombinant mammalian selenoenzymes in heterologous systems, we have optimized the purification of native human thioredoxin reductase. For this purpose, placenta proved to be the organ of choice. The first purification steps involve organic solvents (Table 1). Apart from their antiseptic effect, these solvents denature the bulk of NADP(H)-dependent enzymes (22), which greatly enhances the efficiency of affinity chromatography used in a later purification step. In comparison with the original report of Obholz et al. (5), we were able to improve the isolation procedure with respect to speed, final
The selenium content of the Sephadex G-200 eluate was determined to be 0.94 ± 0.03 mol/mol subunit using atomic absorption spectroscopy. In a number of preparations, the specific activity did not increase in the last step. The value of 35.0 units/mg, corresponding to 2 units/nmol subunit, is not exceeded by any known mammalian thioredoxin reductase. 0.2 mg/ml bovine serum albumin in the assay mixture increased the final specific activity to 42 units/mg. Bovine serum albumin, however, was not used in assay mixtures since it interfered with the studies on tight-binding enzyme inhibitors.

| Purification of a human thioredoxin reductase from 530 g of frozen placenta | mg protein | Total units | Specific activity | Step to step yield | Overall yield |
|---|---|---|---|---|---|
| Chloroform-butanol extract | 6750 | 135 | 0.02 | (100) | (100) |
| Acetone-treated fraction | 2910 | 131 | 0.045 | 97 | 97 |
| DEAE-cellulose eluate | 530 | 60 | 0.195 | 79 | 77 |
| 2',5'-ADP-Sepharose eluate | 1.98 | 67.2 | 33.9 | 65 | 50 |
| Sephadex G-200 eluate | 0.96 | 33.6 | 35.0 | 50 | 25 |

The kinetic parameters were obtained from secondary plots of the steady state kinetic data shown in Fig. 1. As in the case of the closely related enzyme glutathione reductase (7, 12, 25, 26) the results for TrxR are consistent with a ping-pong mechanism. We determined a $k_{cat}$ of 3330 ± 882 min$^{-1}$ and $K_m$ values of 18 μM for NADPH and of 25 μM for E. coli thioredoxin. When using the DTNB reduction assay, the $K_m$ for NADPH was found to be only 6 μM; the $K_m$ value of 0.4 mM for DTNB as reported by Oblong et al. (5) was confirmed. Thus, our results obtained with the DTNB reduction assay compare well with a previous study on human placenta TrxR (5) and with the data for TrxR from rat liver (24) and mouse tumor cells (3, 27).

It should be noted, however, that at NADPH concentrations above 20 μM, substrate inhibition becomes appreciable in both assay systems. This is apparent in Fig. 1 where the data points depart from the straight lines at higher concentrations of NADPH.

The effect of temperature on the rate of the reaction was studied both with TrxS$_2$ and with DTNB as a substrate; the data for E. coli TrxS$_2$ are given in Fig. 2. In the range between 5 and 40 °C, the activation energy was found to be 53.2 kJ/mol. Above 40 °C, human TrxR becomes unstable and is completely inactive at 60 °C. Glutathione reductase and other disulfide reductases are known to be fully active at this temperature (6, 28, 29).

Using the Arrhenius plot (Fig. 2), the turnover number at 4 °C is 650 ± 17 min$^{-1}$ or 10.8 ± 2.9 s$^{-1}$. As discussed below this value is consistent with the slowest step in the reductive half-reaction determined at 4 °C in an earlier study of the pre-steady state kinetics (7).

Inhibitor Studies—The results of the studies with aurothioglucose and auranofin in the assay mixture are shown in Fig. 3. Gold-free thioglucose did not inhibit the enzymes in the concentration range used for the gold compounds. Glutathione reductase and glutathione peroxidase were by at least three orders of magnitude less susceptible to the organic gold compounds than thioredoxin reductase. For glutathione peroxidase, the situation was reversed; that is, auranofin but not aurothioglucose was found to be an inhibitor. Qualitatively speaking, our data agree well with the effects of...
almost completely inhibited (Fig. 4). The enzymes remained unaffected, NADPH-reduced TrxR was inhibited by 50% by 100-fold less gold compound (15). Due to the fact that the compounds have inhibitory effects at concentrations almost equimolar to TrxR, the IC$_{50}$ values presented here can vary depending on the enzyme concentration in the assay (see text).

**TABLE II**

| Inhibitory effects of aurothioglucose (A) and auranofin (B) on three different antioxidant human enzymes. Concentration-dependent inhibition of 3 nM human glutathione peroxidase, 1.5 nM human glutathione reductase, and 2 nM human thioredoxin reductase, respectively, is given for standard assay conditions. The inserts show the chemical structure of the inhibitors. |
|---------------------------------|-----------------|
| Thioredoxin reductase | 65 nM | 20 nM |
| Glutathione reductase | 100 μM | 40 μM |
| Glutathione peroxidase | 80 μM | 100 μM |

Aurothioglucose

**Fig. 3.** Inhibitory effects of aurothioglucose (A) and auranofin (B) on three different antioxidant human enzymes. Concentration-dependent inhibition of 3 nM human glutathione peroxidase, 1.5 nM human glutathione reductase, and 2 nM human thioredoxin reductase, respectively, is given for standard assay conditions. The inserts show the chemical structure of the inhibitors.

**Aurothioglucose**

- hGPx
- hTrxR
- hGR

**Auranofin**

- hGPx
- hTrxR
- hGR

Due to the fact that the compounds have inhibitory effects at concentrations almost equimolar to TrxR, the IC$_{50}$ values presented here can vary depending on the enzyme concentration in the assay (see text).

| IC$_{50}$ values of the inhibition of human glutathione reductase, glutathione peroxidase and thioredoxin reductase by different gold compounds |
|---------------------------------|-----------------|

**Fig. 4** shows the different sensitivity of isolated TrxR and glutathione reductase in their oxidized (E$_{ox}$) and NADPH-reduced states toward gold compounds. Whereas in the E$_{ox}$ form the enzymes remained unaffected, NADPH-reduced TrxR was almost completely inhibited (Fig. 4A). The addition of 1 mM BAL reversed the TrxR-inhibition, leading to 50% of initial activity after 5 min and 100% after 20 min. Simultaneous incubation with inhibitor and BAL completely prevented the effect of the gold compounds on TrxR. Glutathione reductase, even in the NADPH-reduced form, was hardly affected by pre-incubation with organic gold compounds (Fig. 4B). Only inorganic Au(III)/Cl$_3$ led to an inactivation of reduced glutathione reductase; in contrast to the situation with human TrxR, this inhibition was only partially reversible by BAL (less than 15% after 1 h).

In an attempt to describe the inhibition of TrxR by auranofin more precisely, we extended this study to include inhibition steady state kinetics for tight binding enzyme inhibitors that compete with a substrate (30). Initially we tried to analyze progress curves as defined by Morrison and Walsh (30). However, assays with different concentrations of both enzyme and inhibitor did not generate the unique curvature required for such a biphasic analysis. Thus, our rate measurements refer to the first minute of an enzyme assay that showed no subsequent curvature.

The data in Fig. 5 were fitted satisfactorily to either Equation 1, representing classical competitive inhibition, or to Equation 2, which accounts for the change in concentrations of inhibitor and enzyme as a result of the tight binding in the enzyme inhibitor complex (30).

\[
\begin{align*}
\bar{v} &= (V_{\text{max}}/K_m)(1 + [I]/K_i) + [S] \\
\bar{v} &= \frac{k_2 [S] (K_m + [I]) (K_i + [I])^2 + 2K_i - [I] [E_i] + [E_i] [I]^2} {([E_i] [I])^2 - (K_1 + [I] - [E_i])} 
\end{align*}

\tag{Eq. 1}

\tag{Eq. 2}

Fitting the experimental data of Fig. 5 to Equation 1 yielded auranofin $K_i$ values of 2.6 nM at [S] = 50 μM TrxS$_2$, of 4.8 nM at [S] = 75 μM TrxS$_2$, and of 0.25 μM for TrxS$_2$ values of $\approx$2500 min$^{-1}$. The value of 2500 min$^{-1}$ for $V_m$ is lower than the $k_{cat}$ of 3300 min$^{-1}$ (see above) because the inhibitor assays were carried out in the presence of 100 μM NADPH where substrate inhibition is appreciable (Fig. 1). The $K_m$ value for E. coli TrxS$_2$ is 25 μM; [I] in Equation 1 was assumed to be the concentration of free inhibitor.

The fitted lines in Fig. 5, however, represent Equation 2, where [S] is the E. coli TrxS$_2$ concentration. Solving Equation 2 for $K_i'$ gave values of 6.0 nM for 50 μM TrxS$_2$ and of 12.0 nM for 75 μM TrxS$_2$; as above, $k_7 = V_m$ was taken to be 2500 min$^{-1}$ and $K_m$ to be 25 μM, whereas $|E_i|$ was 1.73 nM hTrxR subunits. $|I|$ in Equation 2 was assumed to be the total inhibitor concentration. $K_i'$ is an apparent quantity (30) that is related to $K_i$ as given in Equation (3).

\[
K_i' = K_i'/[1 + [S]/K_m]
\tag{Eq. 3}
\]

The $K_i$ values derived from Equation 3 are therefore 2.0 and 3.0 nM at 50 and 75 μM TrxS$_2$, respectively. Thus in our case, Equations 1 and 2 yield very similar results; Equation 2 is particularly relevant when [I] is similar to [E] because it
accounts for the change in concentrations of these quantities during the assay.

We were unable to determine whether there is an additional isomerization complex E-I*; the reaction sequence E → I → E-I* is often to be considered for quantifying the effects of reversible inhibitors with Kᵢ-values in the submicromolar range (30).

The Kᵢ values determined above are approximately 10-fold lower than the IC₅₀ value listed in Table II, which was determined using DTNB as the disulfide substrate. If we assume that DTNB competes with auranofin as is the case for TrxS₂ (Fig. 5), we can apply Equation (4), taking into account the actual DTNB concentration of 3 mM in the assay and its Kₘ of 0.4 mM.

\[ Kᵢ = IC₅₀/(1 + [S]/Kₘ) = 20 \text{ nM}/(1 + 3 \text{ mM}/0.4 \text{ mM}) = 2.4 \text{ nM} \quad (\text{Eq. 4}) \]

Thus, there is good agreement between the Kᵢ values evaluated with TrxS₂ as the substrate (Fig. 5) and the IC₅₀ value evaluated from the DTNB-based assay.

**DISCUSSION**

Isolation of Authentic hTrxR—Difficulty in preparing recombinant hTrxR is a major problem in studying the human thioredoxin system. The published purification protocols for placenta hTrxR result in comparatively low yields and require many time consuming steps (5). The efficient purification procedure presented here provides sufficient amounts of thioredoxin reductase for structural studies. We recently succeeded in crystallizing the isolated enzyme by using PEG 8000 in Tris buffer of pH 7.4 as a precipitant.

Kinetic Studies—The steady state kinetic data are consistent with a bi-bi-ping-pong mechanism, a result which further underlines the similarities between human thioredoxin reductase and glutathione reductase (7, 12, 26). Using the Arrhenius diagram (Fig. 2), the apparent turnover number of hTrxR at 4 °C is 650 ± 17 min⁻¹ or 10.8 ± 2.9 s⁻¹. This allows the comparison of the steady state kinetic data with the pre-steady-state rates determined at 4 °C for the reduction of hTrxR by its substrate NADPH (7); the low temperature was necessary because the first two phases of this reaction are very fast. A rapid absorbance increase at 540 nm (110 s⁻¹), for instance, reflected the reduction of the active site disulfide Cys-57/Cys-62 as indicated by a charge transfer complex between the nascent thiolate S⁻ and FAD. The slowest reaction phase was observed as an absorbance decrease at 540 nm, signaling reformation of the active site disulfide; it occurred at a rate of approximately 5 s⁻¹, which is comparable with the turnover number of TrxR at 4 °C (Fig. 2). On the basis of these data, it is tempting to speculate that kₗ of human TrxR is limited at least in part by redox interchange between the active site Cys-57/Cys-62 pair and the Cys-495/SeCys-496 redox center. The notion that the active site dithiol passes the reducing equivalents on to another redox center is supported by the observation that at least two equivalents of reducing agent (carrying four electrons) are

![Fig. 4. NADPH dependence of TrxR and GR inhibition by different gold compounds. Only the NADPH-reduced form of human thioredoxin reductase (A) is inhibited by 20 min preincubation with 1 μM inhibitor, whereas the enzyme in the Eox form remains stable. In contrast, human glutathione reductase (B) is not affected by aurothioglucose or auranofin under these conditions. However, AuCl₃ does also strongly inhibit the NADPH-reduced form of hGR, indicating a different modification caused by Au(III) when compared with organic Au(I)-compounds.]

![Fig. 5. Inhibition of human thioredoxin reductase by auranofin. The assays were carried out as described under “Experimental Procedures.” The thioredoxin concentrations were 50 μM (triangles) and 75 μM (squares). The data were fitted according to Morrison and Walsh (30).]
Inhibition of Thioredoxin Reductase by Gold Compounds

needed for the complete reduction of the active site disulfide (7).

Gold Compounds as Inhibitors—Aurothioglucose and auranofin were found to be potent inhibitors of human thioredoxin reductase (Table II, Equations 2 and 3). These organic gold compounds are widely used in the treatment of rheumatoid arthritis. The disease is considered to be an autoimmune condition initiated by various agents, the Epstein-Barr virus being the prime candidate (31). Lymphocytes infected with EBV or other viruses have been shown to secrete thioredoxin (32) which, together with our data, suggests the possibility that the thioredoxin redox system plays a prominent role in autoimmune processes. This notion is supported by the finding that in Sjögren’s syndrome, another autoimmune disease with joint involvement, secreted thioredoxin levels correlate very well with the expression of EBV material (34). With respect to the inhibition studies on TrxR, it should be emphasized that the activity of thioredoxin as a cytokine depends on its reduced dithiol state (32, 33).

As shown in Fig. 4, NADPH-reduced human thioredoxin reductase is highly sensitive to gold compounds, whereas the oxidized form of the enzyme, $E_{ox}$, is not affected. Since the $K_m$ value (as $K_{man}$ value) for NADPH under quasi in situ conditions is $3–5$ times lower than the cytosolic NADPH concentration (7), the reduced gold-sensitive forms of TrxR are likely to be predominant in situ. Not only chemical but possibly also steric reasons may account for the different sensitivities of oxidized and reduced TrxR. For the three human iodothyronine deiodinases, all of them being selenoenzymes, it has been shown that the type I enzyme is strongly inhibited by aurothioglucose ($K_{app} \sim 5 \text{ nM}$), whereas the type 2 and type 3 enzymes are 1000-fold less sensitive (38).

Several lines of reasoning indicate, that it is indeed the gold content of the compounds which leads to thioredoxin reductase inhibition. First, the thioglucose moiety of aurothioglucose (and auranofin) is not an inhibitor in the concentration range used in our study. Second, the gold-chelating agent BAL is able both to prevent and to reverse the inhibition of TrxR caused by three different compounds that have only the gold moiety in common. Furthermore, selenols exhibit a higher tendency to bind heavy metal ions than thiols do (16). It is therefore tempting to propose that in situ, selenides, all of them being selenoenzymes, it has been shown that the type I enzyme is strongly inhibited by aurothioglucose ($K_{app} \sim 5 \text{ nM}$), whereas the type 2 and type 3 enzymes are 1000-fold less sensitive (38).

Several lines of reasoning indicate, that it is indeed the gold content of the compounds which leads to thioredoxin reductase inhibition. First, the thioglucose moiety of aurothioglucose (and auranofin) is not an inhibitor in the concentration range used in our study. Second, the gold-chelating agent BAL is able both to prevent and to reverse the inhibition of TrxR caused by three different compounds that have only the gold moiety in common. Furthermore, selenols exhibit a higher tendency to bind heavy metal ions than thiols do (16). It is therefore tempting to propose that...

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