Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 1910–1914, March 1999
Biochemistry

Selenium redox biochemistry of zinc–sulfur coordination sites in proteins and enzymes

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ABSTRACT **Selenium has been increasingly recognized as an essential element in biology and medicine. Its biochemistry resembles that of sulfur, yet differs from it by virtue of both redox potentials and stabilities of its oxidation states. Selenium can substitute for the more ubiquitous sulfur of cysteine and as such plays an important role in more than a dozen selenoproteins. We have chosen to examine zinc–sulfur centers as possible targets of selenium redox biochemistry. Selenium compounds release zinc from zinc/thiolate-coordination environments, thereby affecting the cellular thiol redox state and the distribution of zinc and likely of other metal ions. Aromatic selenium compounds are excellent spectroscopic probes of the otherwise relatively unstable functional selenium groups. Zinc-coordinated thiolates, e.g., metallothionein (MT), and uncoordinated thiolates, e.g., glutathione, react with benzeneseleninic acid (oxidation state +2), benzeneselenenyl chloride (oxidation state 0) and selenocystamine (oxidation state −1). Benzeneseleninic acid and benzeneselenenyl chloride react very rapidly with MT and titrate substoichiometrically and with a 1:1 stoichiometry, respectively. Selenium compounds also catalyze the release of zinc from MT in peroxidation and thiol/disulfide-interchange reactions. The selenoenzyme glutathione peroxidase catalytically oxidizes MT and releases zinc in the presence of *t*-butyl hydroperoxide, suggesting that this type of redox chemistry may be employed in biology for the control of metal metabolism. Moreover, selenium compounds are likely targets for zinc/thiolate coordination centers *in vivo*, because the reactions are only partially suppressed by excess glutathione. This specificity and the potential to undergo catalytic reactions at low concentrations suggests that zinc release is a significant aspect of the therapeutic antioxidant actions of selenium compounds in antiinflammatory and anticarcinogenic agents.**

Mammalian metallothioneins (MT) are 7-kDa proteins in which 20 cysteines bind 7 zinc atoms in two clusters, constituting networks of zinc–sulfur interactions unique to biology (1). This unusual coordination has now been explained in terms of a function of MT by the demonstration that the sulfur ligands and a variety of oxidizing agents interact with concomitant release of zinc. Thus, MT is a temporary zinc reservoir, whose metal content is controlled by redox reactions (2). The redox potential of MT allows its ready reactions with mild cellular oxidants. In efforts to elucidate the compounds that might oxidize MT in the cell, we have established that both disulfides (3) and selenium compounds (4) such as ebselen (2-phenyl-1,2-benzisoselenazol-3(2*H*)-one) (5) react with MT resulting in the prompt release of zinc at equimolar amounts of reactants. Thus, selenium can function in the redox regulation of thiols and may have a significant role by interacting with zinc-coordinated cysteines in cellular zinc metabolism. It is an important aspect of this chemistry that selenium compounds can oxidize thiols under reducing conditions such as those found in the cytosol. Selenium compounds are redox fined-tuned owing to the many different oxidation states of selenium as well as the protein environment in which the element resides and in which it is found catalytically active in peroxidative reactions (6), thiol/disulfide interchange (7), and reduction of cytochrome *c* (8) or molecular oxygen to superoxide (9) by thiols. These multiple catalytic potentials may be among the reasons that selenium compounds are so effective while acting at concentrations much lower than the corresponding sulfur analogues.

The chemistry of selenium qualitatively resembles that of its more abundant homologue, sulfur, but jointly encompasses much greater oxidoreductive potential, particularly when combined with zinc. A functional, catalytic role of selenocysteine has been investigated mainly in glutathione peroxidase, where the selenium atom changes its oxidation states in the course of the catalytic cycle. The biochemical potential of selenium compounds to undergo redox reactions with regard to MT as well as the release of zinc from its zinc–sulfur clusters has now been investigated by employing compounds displaying functional selenium groups that have been observed *in vivo*. This has been achieved through relatively stable phenyl derivatives that also serve as UV/VIS spectroscopic probes suitable for the characterization of the time-course of reactions. The present study demonstrates that selenium compounds can serve as rather specific, mild cellular oxidants of MT in an overall reducing environment and that they can act as catalysts for zinc release.

MATERIALS AND METHODS

Materials. Rabbit MT was a gift from G. J. Xu (Shanghai Institute of Biochemistry). Zn_7-MT was prepared from the cadmium-containing form through procedures described recently (10). Glutathione (GSH), glutathione disulfide (GSSG), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), glutathione peroxidase (Gpx), and *t*-butyl hydroperoxide were obtained from Sigma; benzeneselenol, benzeneseleninic acid, benzenesulfinic acid, and benzeneselenenyl chloride were obtained from Aldrich, and 4-(2-pyridylazo)resorcinol (PAR) was obtained from Fluka.

UV/VIS Spectroscopy. Reactions of MT with selenium compounds were monitored by spectral scans using either CARY 1 or 50 UV/VIS spectrophotometers at 25°C. Nitrogen gas-purged buffers were used throughout.

Zinc Release Assay. The release of zinc from MT was measured spectrophotometrically by observing the formation of the zinc–PAR complex (ε_{500} = 65,000 $M^{-1}\cdot cm^{-1}$) (11) at a PAR concentration of 100 μM in 20 mM Hepes-Na^+, pH 7.5.

Peroxidation Assay. Influence of selenium compounds on the reaction of 0.5 μM MT with 500 μM *t*-butyl hydroperoxide was measured spectrophotometrically by observing the forma-

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Abbreviations: MT, metallothionein; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); GSH, glutathione (reduced form); GSSG, glutathione (oxidized form); Gpx, glutathione peroxidase; PAR, 4-(2-pyridylazo)resorcinol; CDNB, 1-chloro-2,4-dinitrobenzene.

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Table 1. Stimulation of zinc transfer from MT to PAR by selenium compounds in the absence and presence of glutathione (GSH)

Se compound	Concentration, μM	Zinc transferred, %	Zinc transferred in the presence of GSH, %
Selenocystamine	50	30	18
Benzeneselenenyl chloride	20	100	17
Benzeneseleninic acid	20	100	25

MT (0.5 μM) was incubated with selenium compounds in 20 mM Hepes-Na^+/100 μM PAR (pH 7.5) in the absence and presence of a 5-fold excess of GSH over selenium compound. Measurements were taken after 1 h.

tion of the zinc–PAR complex at a PAR concentration of 100 μM in 20 mM Hepes-Na^+, pH 7.5.

Assay for Selenol. The formation of selenol (-SeH) was monitored by using 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) as indicator (12) in 20 mM Hepes-Na^+, pH 7.5. CDNB (from a 100 mM stock solution in methanol) was added to the buffer before the reaction between MT and the selenium compound was initiated. A 10-fold excess of GSH over a given selenium compound served as a standard (100%) for the CDNB titration. An extinction coefficient of ε_{400} = 2,000 $M^{-1}\cdot cm^{-1}$ for DNB–selenocysteamine was estimated by reacting 20 μM seleno-DL-cystine with 200 μM GSH for 1 h in the presence of CDNB. The reaction of 200 μM GSH with CDNB was used as a control (12). Similarly, an extinction coefficient was estimated for DNB–benzeneselenol (ε_{400} = 8,700 $M^{-1}\cdot cm^{-1}$).

RESULTS

Phenyl derivatives of selenium, which are more stable than their aliphatic counterparts, were chosen to characterize the reactivity of selenium compounds in different oxidation states with zinc-bound thiolates. The capacity of these selenium compounds to react with zinc–sulfur clusters of MT was evaluated spectrophotometrically with PAR as an indicator for zinc released (4). In all cases, the selenium compounds were more effective than their sulfur analogues in releasing zinc from MT. Uncoordinated thiols tend to be more reactive than zinc-coordinated thiols (4), and they are in excess over the latter in the cell, e.g., where the cellular glutathione concentration is in the range of 0.1–10 mM (13). Therefore, we have investigated to what extent GSH quenches the reactivity of selenium compounds toward zinc–sulfur clusters. Unlike most other oxidative agents, selenium compounds react with MT even in the presence of a 5-fold excess of GSH over the selenium compound (5). In our investigation, this corresponds to a 200- to 500-fold excess of GSH over MT, a ratio similar to that in the cytosol of cells.

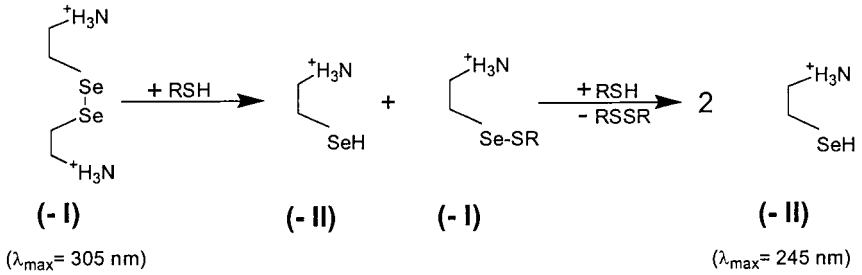
The reduction of selenium compounds by thiols leads to the formation of selenol (-SeH), the reduced functional group of catalytically active selenium compounds (6, 7). Therefore the amount of selenol was determined in the reaction as a measure of the extent of reduction of the selenium compound and its potential to act catalytically. The formation of selenol was found to correlate with the catalytic peroxidation and thiol/disulfide interchange of MT by selenium compounds.

Noncatalytic Reactions Between Thiols and Selenium Compounds. *Oxidation state −1.* Selenium occurs in oxidation states from −2 to +6. Whereas selenium compounds in the most reduced state, −2 (selenol or selenoethers such as benzeneselenol or selenomethionine) do not react with MT, those with selenium in the oxidation states −1 and higher do. Selenocystamine efficiently oxidizes MT and releases 30% of its zinc in 1 h (Table 1), more than twice the amount released under identical conditions by the sulfur analogue, cystamine.

The mechanism for this reaction (Scheme 1) can be deduced from changes in the UV/VIS spectra during the reaction, because the Se—Se bond has a characteristic absorbance band (ε_{300} = 240 $M^{-1}\cdot cm^{-1}$) (14, 15), the absorption maxima shifting to lower energies in the series disulfide → mixed selenodisulfide → diselenide (16). When an equimolar amount of GSH is added to selenocystamine, the absorption at 245 nm increases ($\Delta\varepsilon_{245}$ = 5,500 $M^{-1}\cdot cm^{-1}$) whereas that at 305 nm decreases, indicating the rapid reduction of selenocystamine to selenocysteamine (17, 18) (Scheme 1). The same spectral changes take place with MT, albeit at a much slower rate and with lesser selenol formation under identical conditions. When MT is added in excess (MT thiols/Se = 2:1), only 22% of the amount of selenocysteamine determined in the presence of GSH (GSH/Se = 2:1) is formed (Table 2).

Thus, selenocystamine reacts with MT thiols to form one equivalent of selenol and a mixed selenodisulfide, which then reacts further with thiols to form a disulfide and a second equivalent of selenol. As a result, selenocysteamine [a derivative of selenocysteine, the catalytic amino acid in glutathione peroxidase (Gpx)], is generated *in situ*. Consequently, the reduction of diselenides by MT offers a pathway to generate selenol from relatively stable diselenides.

Oxidation state 0. Benzeneselenenyl chloride reacts with MT to release all zinc atoms for delivery to PAR at a concentration approximately equal to that of MT thiols (Fig. 1 and Table 1). This two-step reaction is considerably faster ($t_{1/2}$ = 5 min) than the reaction with a diselenide and is complete after 20 min. The first step involves the substitution of chloride by thiolate to form a mixed selenodisulfide. The latter then reacts with a second equivalent of thiol to form a disulfide and benzeneselenol (Scheme 2). This mechanism is in accord with the CDNB assay that detects 30% of benzeneselenol theoretically ex-



Scheme 1

Table 2. Catalysis of peroxidation and thiol/disulfide interchange reactions of MT by selenium compounds

Se compound	Oxidation state	Selenol estimated, %	Enhancement of peroxidation,* -fold	Enhancement of thiol/disulfide interchange,† -fold
Benzeneselenol	−2	100	5.3	3.0
Selenocystamine	−1	22	5.7	3.3
Benzeneselenenyl chloride	0	13	6.9	2.5
Benzeneseleninic acid	+2	24	4.3	2.2

For experimental details, see *Materials and Methods*. Se compounds were employed at a concentration of 20 μM. A 2-fold excess of MT thiols over selenium compounds was used. For benzeneseleninic acid, a 4-fold excess of MT thiols was used.
*In the presence of 100 nM selenium compound, 500 μM *t*-butyl hydroperoxide.
†In the presence of 1000 nM selenium compound, 50 μM DTNB.

pected when benzeneselenenyl chloride reacts with a 2-fold excess of GSH, but none when it reacts with an equimolar concentration.

MT reacts differently. The CDNB assay detects <13% of the expected selenol, even when MT thiols are in 2-fold excess (Table 2). In this case, the second step, the possible reaction of $(benzeneselenenyl)_{20}$–MT with MT to form oxidized MT and benzeneselenol, reaches only 13%. The solution becomes turbid owing to the slow precipitation of the resultant benzeneselenenylated MT. Hence benzeneselenenyl chloride reacts with MT thiols in a stoichiometry of 1:1, leading to the formation of benzeneselenenylated MT as the main product of the reaction, which is also in accord with the results obtained by the assay of zinc release (Fig. 1).

Oxidation state +2. Benzeneseleninic acid enhances zinc transfer from MT to PAR at substoichiometric concentrations with respect to thiols of MT and releases all zinc (Table 1). A ratio of MT thiols/Se = 3:1 indicates that one equivalent of benzeneseleninic acid reacts with approximately three MT thiols (Fig. 2). Release of zinc is rapid and proceeds at a rate similar to that with benzeneselenenyl chloride. In contrast, benzenesulfinic acid, a reducing agent, is unreactive.

Theoretically, benzeneseleninic acid could react with four equivalents of thiols to form one equivalent of benzeneselenol and two equivalents of disulfide (combined Schemes 2 and 3; the oxidation state of benzeneselenenyl chloride in Scheme 2 corresponds to that of benzeneselenenic acid in Scheme 3). In accordance with the mechanism proposed (Scheme 3), benzeneselenol is not detected in the presence of an up-to-3-fold excess of GSH, whereas a 4-fold excess leads to 26% benzeneselenol. As in the case of benzeneselenenyl chloride, the major reaction product under conditions of a 4-fold excess of GSH is benzeneselenenyl glutathione, not benzeneselenol.

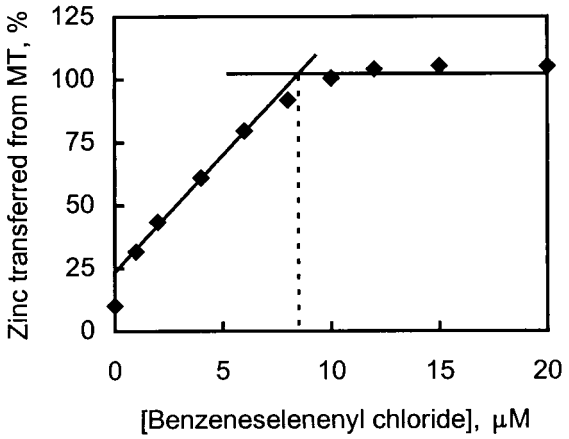


FIG. 1. Concentration dependence of zinc transfer from MT to PAR in the presence of benzeneselenenyl chloride. MT (0.5 μM) was incubated with various concentrations of benzeneselenenyl chloride in 20 mM Hepes-Na^+/100 μM PAR, pH 7.5. Measurements were taken after 1 h.

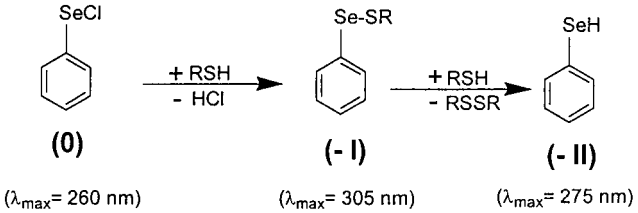
The reaction of benzeneseleninic acid with MT follows a similar pattern. Only 24% of the benzeneselenol theoretically expected is detected even at a 4-fold excess of MT thiols (Table 2). Therefore, three equivalents of MT thiols react with benzeneseleninic acid to form benzeneselenenylated MT (Scheme 2). The latter reacts further with MT only partially to form benzeneselenol, again in agreement with the results obtained for the reaction of MT with benzeneselenenyl chloride.

Steric factors, the poor water solubility of benzeneselenenylated MT, and the lower reactivity of the zinc–sulfur clusters in comparison with uncoordinated thiols account for the suppression of the last step, i.e., the reaction between the selenodisulfide and MT. However, in all of these reactions, selenols are formed, and for this reason, the catalytic effects of these species on peroxidation and thiol/disulfide interchange reactions have been explored further.

Reactions in the Presence of Glutathione. In the presence of a 500-fold excess of GSH over MT, selenocystamine induces zinc transfer from MT to PAR on the order of 60% of that measured in the absence of GSH (Table 1). Thus, GSH does not efficiently quench the reaction of diselenides with MT, suggesting that these agents can act as oxidants even in the reducing environment of the cell. Under similar conditions, GSH quenches the reaction of MT with benzeneselenenyl chloride 83% (Table 1). GSH quenches the reaction with benzeneseleninic acid in a concentration-dependent manner; it is not quenched, however, in the presence of an equimolar or a 2-fold excess of GSH over benzeneseleninic acid. A 3- and 5-fold excess quenches zinc release by 50% and 75% (Table 1), respectively, supporting the conclusion that this selenium compound reacts with at least 3 equivalents of thiols (see above) and that only an excess of GSH quenches its reaction.

Catalytic Reactions Between Thiols and Selenium Compounds. The reaction of MT with the above selenium compounds generates various amounts of selenol (Table 2), a species that catalyzes peroxidation and disulfide interchange of thiols. Hence, these compounds were investigated further with regard to their catalytic properties that result in the release of zinc from MT.

Peroxidation. t-Butyl hydroperoxide (1,000-fold excess) reacts slowly with MT and effects the transfer of only 5% of its



Scheme 2

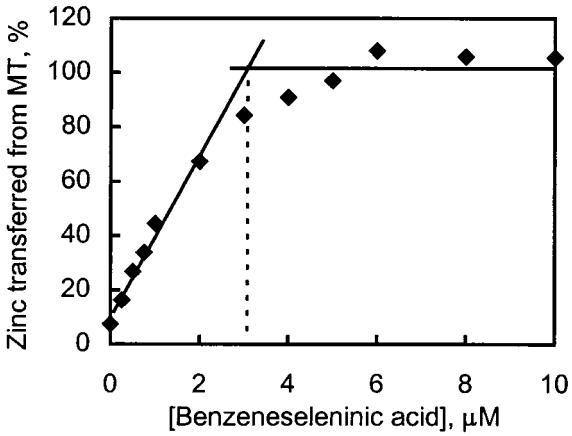
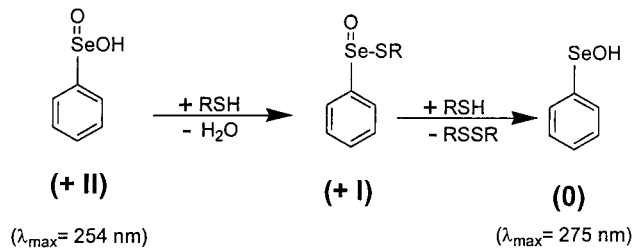


FIG. 2. Concentration dependence of zinc transfer from MT to PAR in the presence of benzeneseleninic acid. MT (0.5 μM) was incubated with various concentrations of benzeneseleninic acid in 20 mM Hepes-Na^+/100 μM PAR, pH 7.5. Measurements were taken after 1 h.

zinc to PAR in 1 h. Catalytic amounts of a selenium compound significantly (4- to 7-fold) enhance this reaction (Table 2). Both benzeneselenenyl chloride and benzeneseleninic acid form catalytic amounts of benzeneselenol (see above), which itself initiates redox cycling of selenium (Fig. 3). In the case of selenocystamine, selenocysteamine is the catalytic species (17) formed by an initial thiol/diselenide interchange of the "procatalyst" selenocystamine with MT. In general, selenol then undergoes peroxidation to form selenenic acid, which is reduced back to selenol in the presence of a 100-fold excess of MT thiols. MT also reduces a disulfide like the sulfur analogue cystamine (19). However, peroxidation of cysteamine will form sulfenic acid, which rapidly forms the disulfide (20). It is this difference between the relative stabilities of selenenic and sulfenic acid that renders selenium compounds more efficient peroxidation catalysts unless the sulfenic acid is stabilized as in peroxiredoxin (21) or other enzymes (20).

When present in the same catalytic amounts as the other selenium compounds, Gpx enhances zinc transfer from MT to PAR 3.8-fold in the presence of *t*-butyl hydroperoxide (Fig. 4). Peroxide first oxidizes the active selenocysteine in Gpx to cysteineselenenic acid, which then oxidizes GSH to glutathione disulfide (GSSG). It has been observed that thiols other than GSH are substrates for Gpx. Thus, thioredoxin serves as a substrate for plasma Gpx (22). Moreover, a membrane-associated Gpx is not specific for GSH and probably uses another thiol substrate (23). Indeed, our results suggest that in catalytic peroxidation, selenocysteine in proteins might use zinc/thiol(ate)s instead of GSH as reducing agents.

Thiol/disulfide interchange. Diselenides increase the rate constant for the reaction between MT and DTNB 3-fold (Table 2). Different rates of the two MT clusters with DTNB (24) are no longer observed. Again, the catalytic species is the selenol, initially formed in the reaction between the diselenide and MT and then oxidized by DTNB to a mixed selenodisulfide. The catalytic cycle is then closed by a reaction of the mixed seleno-



Scheme 3

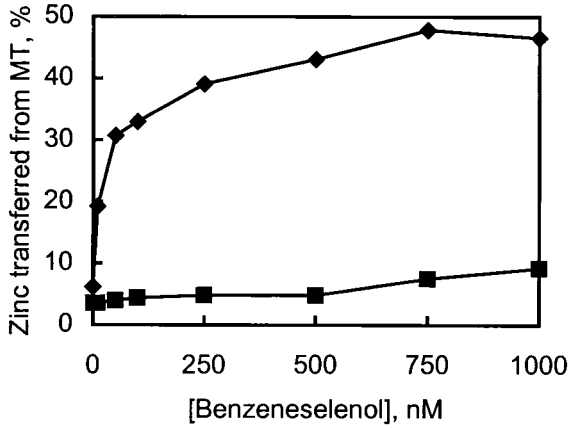


FIG. 3. Zinc transfer from MT to PAR in the presence of *t*-butyl hydroperoxide and benzeneselenol. MT (0.5 μM) was incubated with various concentrations of benzeneselenol in the presence (-◆-) and absence (-■-) of *t*-butyl hydroperoxide (500 μM) in 20 mM Hepes-Na^+/100 μM PAR, pH 7.5. Measurements were taken after 1 h.

disulfide either directly with MT thiols or with another thiol. Further support for the participation of a selenol in the catalytic cycle stems from the observation that benzeneselenol itself increases the rate of thiol/disulfide interchange between MT and DTNB 3-fold (Table 2). Redox cycling between selenenic acid and seleninic acid can be ruled out because DTNB cannot oxidize the former to the latter. Presumably, specific thiol/disulfide interchange is also catalyzed by selenoproteins.

DISCUSSION

Although selenium has been recognized to be an essential micronutrient, the general chemical basis for its role in biochemistry has remained elusive. The redox biochemistry of selenium has been addressed predominantly in relation to the selenocysteine-containing enzyme glutathione peroxidase (Gpx). Neither the precise redox states of selenocysteine during the catalytic cycle of Gpx nor their redox potentials have been determined directly. Most investigations have focused on model compounds of selenium that exhibit peroxidase activity and among those, ebselen is prominent (6). Yet the biological chemistry of selenium is neither limited to its reaction with hydrogen peroxide and GSH nor to the presence of selenocysteine. Glutathione selenotrisulfide, selenophosphate, and methylated selenium compounds, e.g., all occur *in vivo* (25, 26). The most striking feature of the redox chemistry

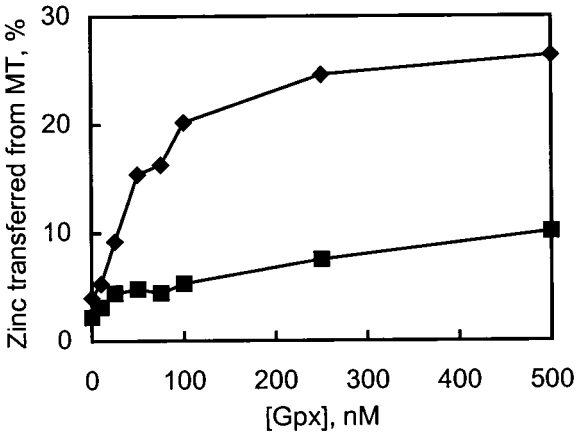


FIG. 4. Zinc transfer from MT to PAR in the presence of *t*-butyl hydroperoxide and Gpx. MT (0.5 μM) was incubated with various concentrations of Gpx in the presence (-◆-) and absence (-■-) of *t*-butyl hydroperoxide (500 μM) in 20 mM Hepes-Na^+/100 μM PAR, pH 7.5. Measurements were taken after 1 h.

of selenium, however, is the interaction with thiols even if and when they serve as metal ligands of zinc. We have now shown that a range of selenium compounds of different oxidation states react rapidly with the zinc–sulfur clusters in MT. The reactivity of benzeneselenenyl chloride, benzeneseleninic acid, and ebselen (5) with MT or GSH far surpasses the reactivity of disulfides or strong oxidizing agents like ferricyanide or hydrogen peroxide.

The redox potential of diselenodiacetic acid is approximately −400 mV vs. the standard hydrogen electrode (27) and, hence, there is little awareness that selenium compounds are oxidants toward thiols. Yet, most of the compounds examined here react specifically with thiols but not with any other amino acid side chain. According to the limited number of redox potentials of selenium compounds that are known, selenol should reduce disulfides, but thiols should not reduce diselenides (7). Apart from questions about the accuracy of these indirectly measured redox potentials, selenium undergoes further reactions that shift the redox equilibria expected in the reactions studied. In catalytic reactions, selenol, e.g., is oxidized easily by traces of oxygen and by *t*-butyl hydroperoxide and disulfides. In such cases, a redox reaction followed by a chemical conversion of one of the products would explain the bias in redox potentials and would allow the reaction to proceed in the direction outlined. Such a mechanism explains the formation of selenol and the consumption of thiols in reactions of MT with selenocystamine, benzeneselenenyl chloride, and benzeneseleninic acid.

The redox chemistry of selenium compounds of oxidation states +2, 0 and −1 with zinc–sulfur clusters identifies these compounds as a prominent group of biological redox catalysts. Selenocysteine and other selenol derivatives undergo rapid peroxidation, forming highly reactive selenenic and seleninic acids. These compounds strongly interact with zinc–sulfur clusters of MT, while in contrast, their corresponding sulfur analogues are considerably less reactive.

Although all selenium compounds investigated also react with GSH, zinc release from MT is observed even if the concentration of GSH exceeds that of the selenium compound 5-fold, corresponding to a 200- to 500-fold excess of GSH over MT. This ratio is similar to that encountered under physiological conditions (13, 28). Apparently, the reactivity of mixed selenodisulfides as reaction products between selenium compounds and GSH is sufficient to oxidize MT thiols. As a consequence, these selenium compounds constitute a class of thiol reagents that have great potential significance for biology because they are highly reactive toward zinc–sulfur clusters under overall reducing conditions and can enter catalytic cycles in the presence of other oxidizing agents. We are unaware of any other class of compounds that exhibits these features to the extent detailed here.

The selenium chemistry cited herein therefore enables the design of redox agents that can target zinc–sulfur bonds, not only in MT but in zinc fingers, zinc twists, and in many transcription factors and signaling proteins. The considerable potential of such compounds as antiviral and anticancer drugs is apparent, and the activity of some selenodrugs based on an interaction with zinc–sulfur centers can be anticipated but has not been systematized (29–31).

There are clearly numerous directions and approaches for the design of reagents and reactions for pharmaceutical exploitation of redox reactions employing the thiol/selenol, disulfide/diselenide and selenenic acid/sulfenic acid oxidation states. They cover a wide range of redox potentials that can be redox fine-tuned further by the protein environment in which they are placed. The redox potentials of biological selenium compounds are sufficiently low and their reactivities sufficiently high that they can and will act as oxidants even in the overall reducing environment of the cytosol. For all oxidation states, highly thiol-reactive species can be designed that can undergo biological redox reactions in conjunction with zinc–sulfur coordination sites.

The close resemblance and interrelationships of the biological chemistries of selenium and sulfur have become apparent only recently as a part of selenocysteine-containing enzymes where selenium plays a pivotal part in the antioxidant defense mechanism of the cell. Peroxides are destroyed by using GSH as an "electron pool." However, the importance of this selenium chemistry for targeting zinc–sulfur clusters and thereby controlling zinc-dependent functions apparently has not been considered. Selenium compounds reduce peroxides while concomitantly releasing zinc very rapidly, thereby suggesting a potential role of selenium in cellular "metal trafficking." Thus, reactive oxygen species generated in oxidative stress release zinc from MT. Both zinc and thionein, T, the apoform of MT, can then function as cellular antioxidants, with the latter then becoming an important endogenous chelating agent of zinc (32). Thus, the oxidant effect of selenium compounds on MT and zinc will likely be part of the antioxidant functions of selenium.

This work was supported by the Endowment for Research in Human Biology, Inc. C.J. holds a Feodor-Lynen Fellowship from the Alexander von Humboldt-Stiftung.

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