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Catalytic Oxidation of Zinc/Sulfur Coordination Sites in Proteins by Selenium Compounds

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ABSTRACT

Zinc/thiolate (cysteine) coordination occurs in a very large number of proteins. These coordination sites are thermodynamically quite stable. Yet the redox chemistry of thiolate ligands confers extraordinary reactivities on these sites. The significance of such ligand-centered reactions is that they affect the binding and release of zinc, thus helping to distribute zinc, and perhaps controlling zinc-dependent cellular events. One new aspect focuses on the thiolate ligands of zinc as targets for the redox action of selenium compounds. A distinctive feature of this chemistry is the capacity of selenols to catalyze the oxidation of zinc/thiolate sites. We here use a chromophoric compound, 2-nitrophenylselenocyanate, to investigate its reaction mechanism with the zinc/thiolate clusters of metallothionein, a protein that is a cellular reservoir for zinc and together with its apoprotein, thionein, is involved in zinc distribution as a zinc donor/acceptor pair. The reaction is particularly revealing as it occurs in two steps. A selenenylsulfide intermediate is formed in the fast oxidative step, followed by the generation of 2-nitrophenylselenol that initiates the second, catalytic step. The findings demonstrate the high reactivity of selenium compounds with zinc/thiolate coordination sites and the potent catalytic roles that selenoproteins and selenium redox drugs may have in affecting gene expression via modulation of the zinc content of zinc finger proteins. Antioxid. Redox Signal. 3, 651–656.

INTRODUCTION

THE SULFHYDRYL GROUP OF CYSTEINE is a common ligand of zinc in proteins. Zinc/thiolate coordination is most prevalent in structural sites of proteins, although it is found in catalytic sites of some enzymes (24). It occurs in all zinc finger proteins, which are abundant and estimated to be encoded by a few percent of the genes in the human genome. Zinc/thiolate coordination sites are thermodynamically quite stable and are therefore well suited to serve as scaffolds for protein domains (23). However, there is growing appreciation that these sites exhibit exquisite chemical reactivities in both metal exchange and ligand-centered reactions (12), and that they are therefore not merely inert, structural features of proteins. For example, oxidation of thiolate ligands is a newly proposed mechanism of mobilizing the biologically redox-inert zinc ion from its binding sites (11). Among the oxidants are biological selenium compounds and selenium redox drugs (10). A remarkable feature of selenium compounds is their capacity to act as catalysts. They catalyze thiol/disulfide interchange reactions that release zinc from zinc/thiolate coordination sites, and they catalyze the reduction of protein disulfides (17), thereby generating potential binding sites for zinc in proteins. These reactions, regardless of whether catalyzed by selenium compounds of low molec-

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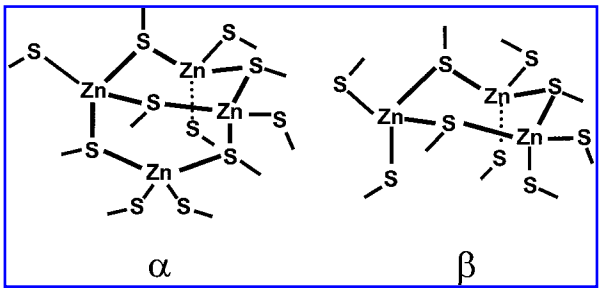


FIG. 1. The two types of zinc/thiolate clusters (α, β) in MT. Every zinc resides in a tetrathiolate coordination environment. As the number of cysteine ligands is less than the 28 ligands that would be required for tetrathiolate coordination of isolated zinc ions, several thiolates form ligand bridges, whereas others coordinate in an end-on fashion.

ular weight or by selenium enzymes, render zinc/thiolate coordination sites susceptible to redox control, a mechanism that might be used physiologically, but certainly can be exploited pharmacologically.

Here, we study the reaction mechanism of a selenium compound with zinc/thiolate centers. Metallothionein (MT) was selected as a target, as it is a major cellular reservoir of zinc and a redox-modulated protein (11, 22). In MT, seven zinc atoms are bound to 20 cysteines in two zinc/thiolate clusters, one with three zincs and nine cysteine ligands, the other with four zincs and 11 cysteine ligands (Fig. 1) (15, 26). An aromatic selenium compound [2-nitrophenylselenocyanate (NPSC); Fig. 2] was chosen because its chromophoric properties make it possible to follow its reactions (10), and because selenocyanates have therapeutic properties. Thus, benzylselenocyanate and various isomers of xylyl-bis(selenocyanate) are active in chemoprevention (3, 6, 14), and potassium selenocyanate has anticarcinogenic activity (9, 21). Our results suggest that a mechanism of the hitherto unknown action of selenium drugs is their interaction with zinc/thiolate coordination sites and concomitant release or binding of zinc.

MATERIALS AND METHODS

Materials

Zn_7-MT-I was prepared from the cadmium-containing form (25). 2-Carboxy-2′-hydroxy-5′-sulfoformazylbenzene (ZINCON), 1-chloro-2,4-dinitrobenzene (CDNB), and selenocystamine were obtained from Sigma, and NPSC from Aldrich.

UV/visible spectroscopy

Reactions of MT with selenium compounds were monitored with a CARY 1 spectrophotometer at 25 or 37°C. Nitrogen gas-purged buffers were used throughout. Kinetic rates were calculated by fitting progress curves with CARY (Varian) software.

Zinc release assay

The release of zinc from MT was measured spectrophotometrically by observing the formation of a zinc-ZINCON (ε_{620} = 175,000 M^{-1} cm^{-1}) complex at a ZINCON concentration of 100 μM in 20 mM HEPES-Na^+, pH 7.4.

RESULTS AND DISCUSSION

Various selenium compounds oxidize the zinc/thiolate clusters in MT and release zinc (10). This process can be followed with chromophoric chelating agents for zinc such as ZINCON and with chromophoric selenium compounds such as NPSC, a stable selenium compound and a precursor for catalytic selenol (18). Here the reaction of MT-I with NPSC was studied, because it proceeds in two phases (Fig. 3) and therefore provides new insights into this novel chemistry.

At a twofold excess of sulfhydryls in MT over selenium (Se:S = 1:2), NPSC releases >80% of the total zinc of MT in 2 h (Fig. 3, trace a). The reaction proceeds in two well separated steps. Up to 40% of the total zinc is released in the fast phase of the reaction that is almost com-

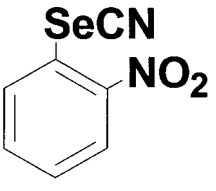


FIG. 2. Chemical structure of 2–nitrophenylselenocyanate (NPSC).

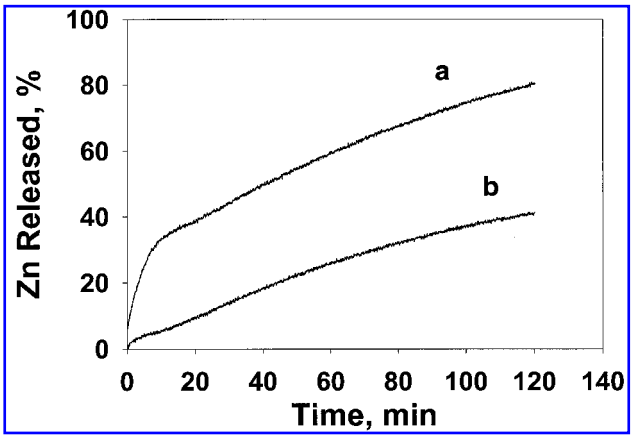


FIG. 3. Kinetics of the reaction between NPSC and MT-I. (**a**) Zinc release from MT-I (0.5 μM) by NPSC (0.5 μM) at a ratio of Se:S = 1:20. (**b**) Zinc release from MT-I (0.5 μM) by NPSC (5 μM) at a ratio of Se:S = 1:2. Zinc release was detected spectrophotometrically by chelation to ZINCON (100 μM) at 620 nm, 37°C. Buffer: 20 mM HEPES-Na^+, pH 7.4.

plete within 20 min before the onset of the second phase. Decreasing the concentration of NPSC (Se:S = 1:10) releases 60% of the total zinc in 2 h, whereas further decreasing the ratio to 1:20 lowers the amount of zinc release to 40% (Fig. 3, trace b). A fast phase that is complete within 10 min can still be detected and leads to a release of ~5% of the total zinc. The amplitude of the first phase correlates with the amount of NPSC added, indicating a stoichiometric reaction in which zinc release from MT is stimulated by oxidation of thiols and -Se-S- (selenenylsulfide) bonds are formed. The percentage of zinc released at the end of the second phase, however, is much higher than expected on the basis of a stoichiometric reaction between selenium and sulfur. Moreover, the rate of this phase does not seem to show a strong dependence on the amount of NPSC. Therefore, this second phase likely represents a catalytic process, in which an adjacent thiolate in MT attacks the reactive -Se-S- bond, releasing selenolate (-Se^-), and forming a more stable disulfide bond (2, 6). Selenolate (selenol) is unstable and easily oxidized to selenenic acid by trace amounts of oxygen (10, 18). Selenenic acid then oxidizes the thiolates of MT to form new -Se-S- bonds. In this manner, a catalytic cycle is sustained by selenolate until all the thiolates in MT are oxidized and all zincs are released. Substoichiometric amounts of selenol therefore play an important catalytic role in the oxidative release of zinc from MT. Corresponding sulfur compounds do not show this catalytic potential. Further support for the reaction mechanism (Scheme I) is provided by the following experiments.

Spectral scans during the reaction between NPSC and MT-I provide insight as to mechanistic details (Fig. 4). MT does not absorb in the near uv region of the electromagnetic spectrum due its lack of aromatic amino acids. The decrease of absorbance at 218 nm, a wavelength at which Zn-S ligand-to-metal charge-transfer bands in MT are measured, coincides with the zinc release from MT as detected by ZINCON. Concomitant increases of absorbance at 252, 308, and 410 nm are assigned to the formation of a -Se-S- bond between NPSC and MT on the basis of the reaction of NPSC with glutathione (Se:S = 1:1) and previous studies of reactions between selenium compounds and MT (10). Because the absorption band with a maximum at 410 nm is well separated from all other absorption bands, it was chosen for kinetic studies. In contrast to the reactions followed by zinc release with ZINCON, -Se-S- bond formation reaches a plateau after ~25 min when stoichiometric amounts of NPSC are used. At higher concentrations of NPSC, all the thiols of MT are converted to -Se-S- bonds, leaving no thiols to displace selenols. At lower concentrations of NPSC (1 μM, Se:S = 1:10), the ab-

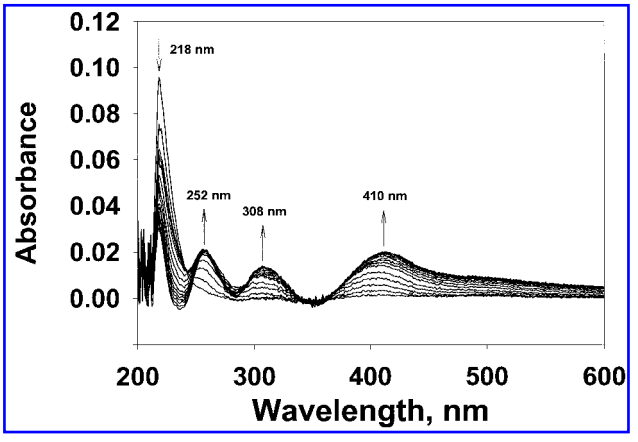
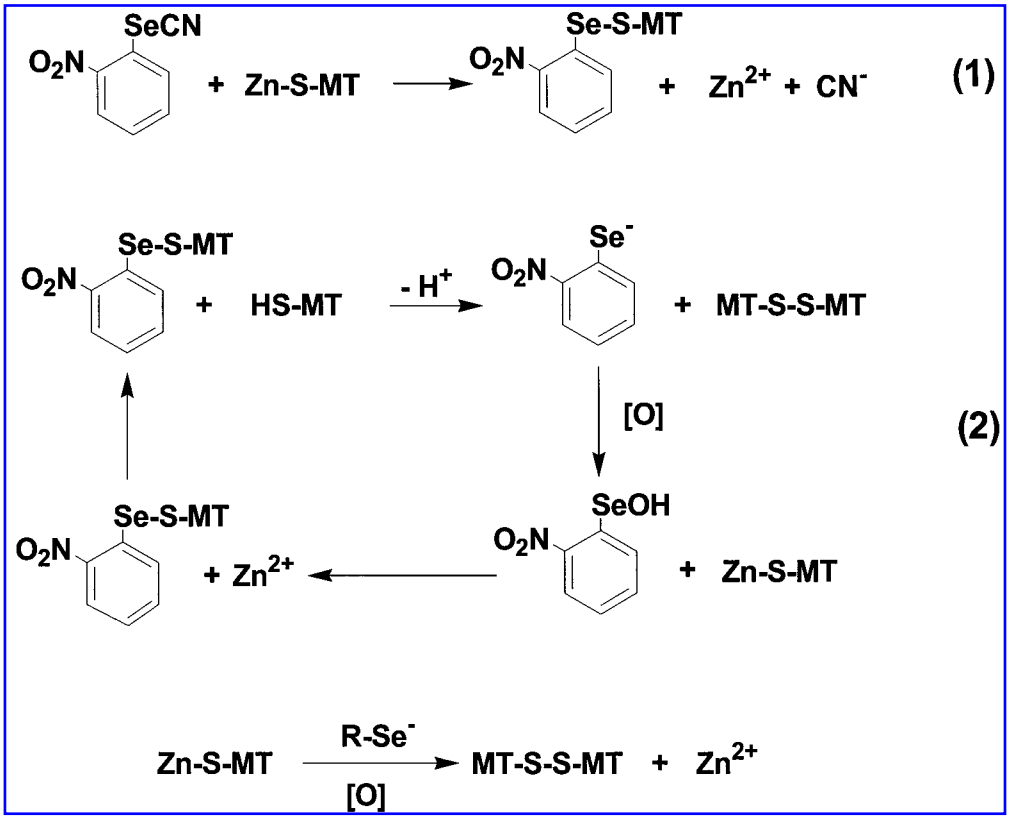


FIG. 4. Spectral scans of the reaction between NPSC (10 μM) and MT-I (0.5 μM). Spectra were recorded every 2 min for 1 h. The reaction was performed in 20 mM HEPES-Na^+, pH 7.4, at 25°C.



Scheme I. Proposed mechanism for the catalytic oxidation of metallothionein by 2-nitrophenylselenocyanate.

sorbance at 410 nm reaches a maximum within several minutes and then gradually decreases due to conversion of -Se-S- bonds to disulfide bonds. A slight red shift of the absorbance at 410 nm during the reaction suggests formation of a new selenium compound, probably a diselenide.

To demonstrate the formation of selenol in the second phase of the reaction, CDNB was added at the beginning of the reaction under

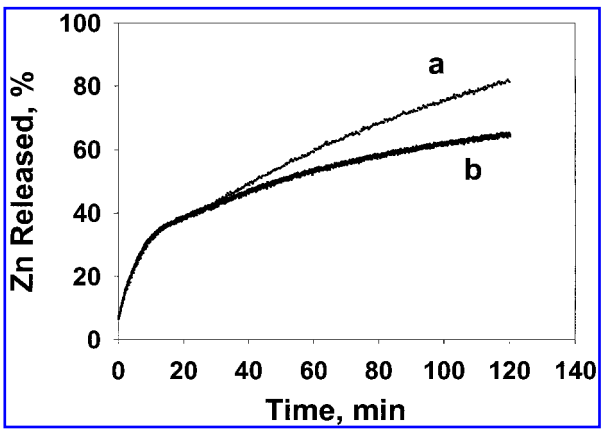


FIG. 5. Kinetics of the reaction between NPSC (5 μM) and MT-I (0.5 μM) without (a) and with (b) CDNB (20 μM) added at the beginning of the reaction. Reaction conditions are the same as those described in the legend of Fig. 3.

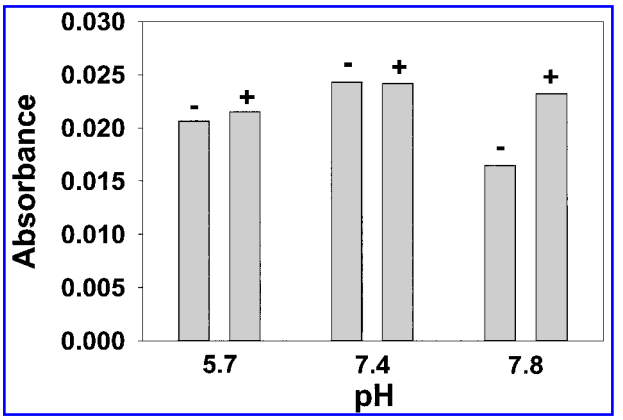


FIG. 6. Effect of pH on the formation of the catalytic selenol in the reaction between NPSC and MT-I. Absorbance readings at 400 nm were taken after 1 h for reactions of NPSC (10 μM) with MT (0.5 μM), at pH 5.7, 7.4, and 7.8 and 25°C. At each pH, samples were prepared with (+) and without (−) added CDNB (1 m*M*).

conditions of substoichiometric amounts of selenium (Se:S = 1:2). CDNB reacts much faster with selenols than with thiols (1) and does not release zinc from MT. Zinc release during the second phase effectively decreases in the presence of CDNB, whereas the first phase is not affected (Fig. 5). After 2 h, 64% of zinc was released in the presence of CDNB (Fig. 5, trace b) compared with 81% in its absence (Fig. 5, trace a).

Reactions of NPSC with MT were also followed spectrophotometrically at pH values of 5.7, 7.4, and 7.8 (Fig. 6). At each pH, samples were prepared with and without CDNB. The basis of this experiment is as follows. If the -Se-S- bond is cleaved into $-Se^-$ and $-S^-$, the sample with CDNB will give a much higher absorbance reading at 410 nm compared with the one without CDNB. A large difference was observed only for the reaction at pH 7.8, indicating that the -Se-S- bond is less stable at high pH and that more selenolate is formed. The pK_a of selenol is ~5.2, whereas that of a thiol is 8 or higher (19). So at higher pH, deprotonation of thiols to thiolates increases their reactivities toward the -Se-S- bonds and disulfide bonds are formed preferentially. Selenotrisulfides illustrate this behavior. They are formed most efficiently under acidic conditions, whereas at pH 7 or higher they undergo rapid decomposition to a disulfide and selenium (4, 16).

Implications

Important aspects of selenium biology are the potency of selenium compounds (5, 8) and the rather narrow window between essentiality and toxicity of selenium. These aspects can be understood on the basis of the catalytic potential of selenols, a potential that now includes reactions affecting the binding and release of zinc.

The redox chemistry studied here underscores the remarkable reactivity of zinc/thiolate coordination sites in proteins with selenium compounds. Selenols, or selenium compounds that serve as precursors of selenols, catalyze the oxidation of zinc/thiolate sites with concomitant release of zinc (10). Selenols also catalyze the reduction of disulfides (17). Reduction of disulfide bonds in proteins potentially regenerates zinc-binding sites. Selenium compounds oxidize thiolates in the presence of a large excess of glutathione, suggesting that these reactions can occur in the overall reducing cellular environment (10). The relevance of this chemistry for pathophysiology, pharmacology, and toxicology is apparent. Selenium redox drugs for antioxidant, antiinflammatory, anticarcinogenic (chemopreventive), and antiviral purposes might all affect zinc finger structures of critical protein targets in signal transduction or gene transcription. At present, the specific circumstances under which selenium redox catalysis controls the zinc content of proteins *in vivo* are unknown. Protein kinase C is a zinc protein from which zinc might be released in such reactions (7). It is known, however, that thiol-reactive oxidants such as selenite increase the concentration of freely available zinc in whole cells (20). Changing the amount of available zinc affects many cellular parameters. For example, zinc in the low nanomolar range inhibits enzymes involved in energy metabolism and in signal transduction (13). In essence, this coupling between sulfur and selenium redox chemistry turns selenium compounds into potent effectors of zinc-dependent cellular events.

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ABBREVIATIONS

CDNB, 1-chloro-2,4-dinitrobenzene; MT, metallothionein; NPSC, 2-nitrophenylselenocyanate; ZINCON, 2-carboxy-2′-hydroxy-5′-sulfoformazylbenzene.

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