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Communication

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Density Functional Theory Study of the Attack of Ebselen on a Zinc-Finger Model

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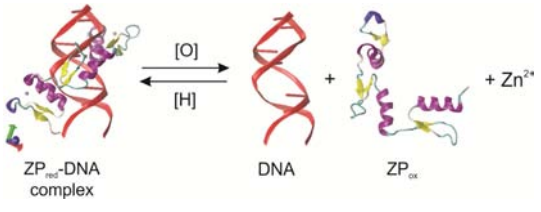
Supporting Information

ABSTRACT: Density functional theory and solvent-assisted proton exchange are used to model the attack of ebselen **1** on a zinc-finger model, an important step in the regulation of zinc signaling by reducible selenium compounds. These calculations show that the formation of a selenosulfide bond from an Se···S intermediate complex between **1** and a Cys_2His_2 zinc-finger model can occur through a moderate activation barrier that is consistent with experimental observations of the relative rates of Zn^{2+} release from zinc-finger transcription factors and metallothionein.

Redox signaling by zinc−sulfur proteins (ZPs) is important to nucleic acid transcription, recognition, and repair; protein regulation; and zinc storage and metabolism.[1−6] Zinc-finger (ZF) transcription factors incorporate Zn^{2+} ions tetrahedrally coordinated to Cys and His residues (typically Cys_2His_2, Cys_3His, or Cys_4) to ensure proper folding of the ZF tertiary structure for biological recognition. The redox activity of the Cys thiolates creates a variable environment for Zn^{2+} binding or release that is critical for the control of transcription, recognition and other mechanisms of cellular signaling ("zinc switch").[3,5,7] The Zn^{2+} ion coordinates to a ZP in its reduced state (ZP_{red}) in which all Cys's are in the thiolate form. Altering the Cys oxidation state by biological or xenobiotic oxidants (H_2O_2, NO, *S*-nitrosothiols, etc.) releases Zn^{2+} with a subsequent loss of the tertiary structure in ZP_{ox} necessary for recognition (Scheme 1).[3,5,7] Reduction of ZP_{ox} with thiols restores the ability

Scheme 1. Representation of the "Zinc Switch" for a ZF-Type ZP (Adapted from PDB ID 1TF3)

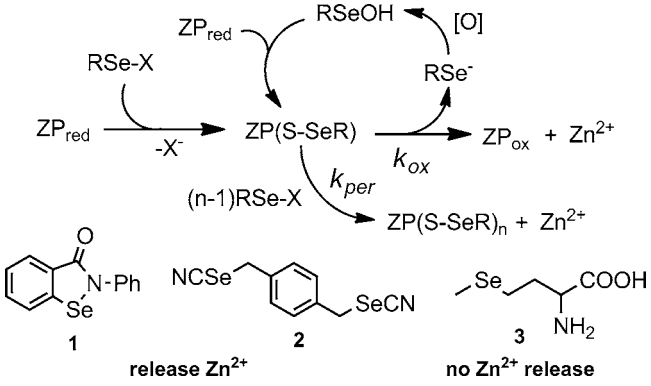


to bind Zn^{2+}. Targeting of ZPs is important to potential treatments of viral infections and cancer by the disruption of gene expression and DNA repair,[8] but may prevent normal repair of damaged DNA and impair genomic stability.[9]

Ebselen **1** and other reducible selenium (rSe) compounds have been shown to release Zn^{2+} from various ZPs.[9−15] Here, rSe compounds are defined as selenium compounds not in the lowest Se(−2) oxidation state: selenite, seleninic acids $RSeO_2H$, and divalent organoselenium compounds RSeX (e.g., **1**, diselenides RSe−SeR′, selenosulfides RSe−SR′, selenenyl chlorides RSe−Cl, and selenocyanates RSe−CN such as the antitumor agent 1,4-phenylenebis(methylene)selenocyanate **2**[16]). In contrast, fully reduced selenols and selenides, such as selenomethionine **3**, do not affect Zn^{2+} release.[9] **1**, a well-known antioxidant mimic of the selenoprotein glutathione peroxidase,[17,18] inhibits DNA binding to transcription factor IIIA[13] and releases Zn^{2+} from the Sp1 transcription factor[13] (Cys_2His_2 type), the formamidopyridine−DNA glycosylase[9] and xeroderma pigmentosum group A[19] (Cys_4 type) repair proteins, as well as metallothionein (MT),[11] the $Zn(Cys_3His)$ site of a histone lysine demethylase[20] and an alcohol dehydrogenase with two Zn−S centers.[11] Although **1** and other rSe compounds are often considered antioxidants, Zn^{2+} release is an important prooxidant mechanism.

Experimental studies of Cys_2His_2- and Cys_4-type ZPs suggest two mechanisms for reaction with divalent rSe compounds RSeX, which have been combined in Scheme 2. Zn^{2+} can be

Scheme 2. Mechanisms of Zn^{2+} Release from ZPs by Divalent Reducible Organoselenium Compounds (RSeX)



released either by oxidation of the thiolates to disulfides (ZP_{ox}) or by perselenenization of the Cys residues [$ZP(S-SeR)_n$], as observed for MT ($n = 20$).[19,21] The rSe compound first reacts to form an Se−S bond through electrophilic attack on one of the Cys residues. From this monoselenenated intermediate ZP(S−SeR), the ZP can either eliminate selenolate RSe^- by forming a disulfide bond (ZP_{ox}) or additional equivalents of RSeX can react with the remaining Cys ligands [$Zn(S-SeR)_n$]. Either oxidative

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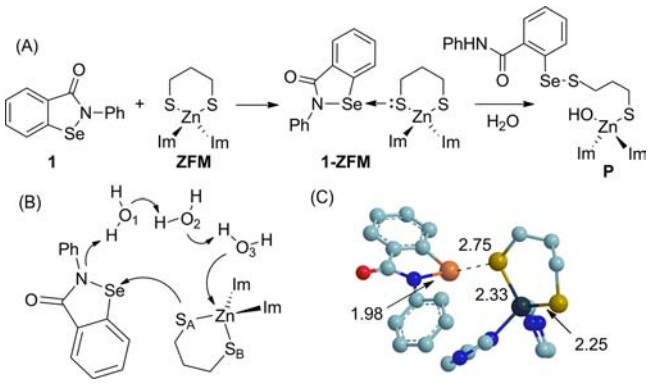
modification weakens the affinity of zinc for sulfur to facilitate Zn^{2+} release. Pathways that result in oxidized ZPs with combinations of disulfide and selenosulfide bonds as discussed for Zn^{2+} release from the NCp7 nucleocapsid (Cys_3His type) by a disulfide can be drawn[22] but have not been reported for rSe compounds. Selenolates produced in the formation of ZP_{ox} can be oxidized to the selenenic acid RSeOH to catalyze Zn^{2+} release. Because free Zn^{2+} is associated with gene expression, apoptosis, and cell growth,[23,24] it is important to understand the redox chemistry of ZFs with rSe compounds.

In this Communication, we use density functional theory (DFT; see the Supporting Information for details) to model the formation of the selenosulfide intermediate from the attack of **1** on a model with a coordination sphere similar to that of a Cys_2His_2 ZF, the most common ZP motif, (Scheme 3) using

Scheme 3. (A) Formation of an Initial Se···S Donor–Acceptor Intermediate 1-ZPM as Part of the Attack of 1 on a Cys_2His_2 Model ZFM, (B) Bond Formation Pathway for the SAPE Model of the Reaction (A), and (C) Structure of 1-ZPM (Bond Distances in Angstroms)



solvent-assisted proton exchange (SAPE). This technique of including explicit protic solvent molecules to shuttle protons in a reactive gas-phase model has been used previously to explore redox scavenging by **1**.[25] The ZF model (**ZFM**) replaces His and Cys with imidazole (Im) and 1,3-propylenedithiolate, respectively. The thiolates have been tethered to represent the 2–5 hydrophobic amino acids that provide structural stability to the ZF[26,27] and to prevent the selenosulfide from drifting away from the zinc coordination site during the DFT study. Formation of the Se–S bond is expected to be rate-determining for Cys_2His_2-type ZFs because the oxidized protein ZP_{ox} is obtained from the reaction of **1** with a fragment of Sp1.[13]

The reactant complex **RC** for the DFT-SAPE study was constructed by adding a three-water network to the donor–acceptor complex of **1** with **ZFM** (**1-ZFM**, Scheme 3). These water molecules facilitate protonation of the amide leaving group of **1** required to break the Se–N bond and provide a hydroxide ligand to complete the coordination sphere of Zn^{2+} (Scheme 3B). **1-ZFM**, in which the ZFM forms an Se···S chalcogen bond with RSeX, is assumed to be an initially formed intermediate in selenosulfide bond formation (Scheme 3A). Donation of a thiolate sulfur lone pair to the antibonding Se–N molecular orbital of **1** results in a strong Se···S interaction (2.75 Å), as indicated by natural bond orbital[28] donor–acceptor calculations ($\Delta E_{d\rightarrow a}$ = 36.2 kcal/mol). Surprisingly, Se···S interactions between rSe compounds and ZFM are stronger than those with a simple thiol because of destabilization of the sulfur lone pairs through metal coordination.[19] Complexation weakens the Zn–S bond (2.33 Å relative to 2.27 Å in **ZFM**) and activates the Se–N bond (1.98 Å relative to 1.88 Å in **1**) toward nucleophilic substitution. The increase in the partial negative charge of nitrogen [−0.75e (**1**) vs −1.21e (**1-ZFM**)][29] facilitates proton transfer to the activated Se–N amide.

The structure of **RC** (Figure 1) was optimizing using DFT(mPW1PW91[30]) and either the B2 all-electron basis set[31] (BSL) or the small-core Ermler–Christiansen relativistic effective core potential (RECP) basis set[32,33] (BSEC) for zinc. The three water molecules of the SAPE network extend from the amide nitrogen to terminate in a hydrogen-bonding interaction with CH groups from each Im rather than interacting directly with zinc [$d_{Zn\cdots O}$ = 4.74 (BSL) and 4.72 (BSEC) Å]. [Note that if the Wadt–Hay large-core RECP basis set[35] is used for zinc (called by either LANL1DZ or LANL2DZ in the Gaussian[36] packages), the **RC** optimizes to a five-coordinate, trigonal-bipyramidal geometry around zinc incorporating a water molecule from the SAPE network as the fifth ligand. See Supporting Information.] From **RC**, cleavage of the activated Se–N bond requires transfer of a proton from the SAPE network to the amide nitrogen. The concurrent formation of the Se–S bond oxidizes and neutralizes the thiolate, which moves away from the coordination sphere of Zn^{2+} to be replaced by an OH^- group formed by proton transfer to the amide nitrogen from the SAPE network. The DFT(mPW1PW91)/BSEC transition state (**TS**) for this process (Figure 1A) is found where the Se–N bond has increased by 0.71 Å and the Se–S bond has decreased by 0.40

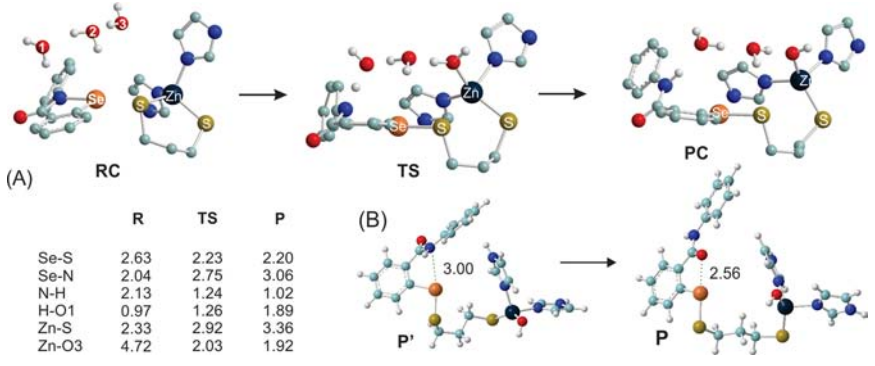


Figure 1. (A) Selected DFT(mPW1PW91)/BSEC bond distances (Å) for the attack of ebselen **1** on a model of a Cys_2His_2 ZF. Hydrogen atoms not involved in the SAPE network have been removed for clarity. (B) Structure for the rearrangement of the selenosulfide product from **P** (Se···N) to **P′** (Se···O).

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Å. The OH^- group, outside the coordination sphere of Zn^{2+} in **RC**, moves close to the metal (2.03 Å) at the **TS** as a Zn−O bond begins to form. The replacement of thiolate in the coordination sphere of Zn^{2+} by OH^- is an important feature of the SAPE model. The solvation-corrected energy of the reaction for the product complex **PC** is endothermic (ΔG = 3.1 kcal/mol). In **PC**, the selenosulfide **P′** forms an Se···N interaction with the amide group that can rearrange to interact with the more basic carbonyl oxygen[29] (Se···O short contact), as shown for **P** (Figure 1B), which is 2.7 kcal/mol more stable. Further oxidation to the disulfide through attack of the remaining thiolate on the selenosulfide is a unimolecular process and is expected to be rapid from this point. The loss of both thiolates from the coordination sphere of Zn^{2+} allows its release and the unraveling of the ZF tertiary structure required for DNA recognition.

The solvation-corrected activation barrier ($\Delta G^{\ddagger}$ = 15.6 kcal/mol) is lower than the DFT barrier to H_2O_2 oxidation of a similar ZFM (20.9 kcal/mol)[34] due to the softness of the selenium electrophile. Experimental studies show that **1** reacts more slowly with MT, which coordinates Zn^{2+} through Cys only and is thus more nucleophilic than a Cys_2His_2 ZF, than with a simple thiol like glutathione [GSH; $t_{1/2}$(GSH) ≈ 5 ms vs $t_{1/2}$(MT) ≈ 5 s].[11] The DFT barrier for our Cys_2His_2 ZFM is higher than the reaction of MeSH with **1** (8.4 kcal/mol),[25] which agrees with a faster reaction with thiols relative to ZFs. Because Cys_3His- and Cys_4-type ZFs are more nucleophilic, their barriers for Se−S bond formation are expected to be lower than that demonstrated here for a Cys_2His_2 ZF-like model. The perselenenization of MT by **1** is complete within seconds,[11] whereas Zn^{2+} release from Sp1 via disulfide formation is only 50% complete after 30 min.[13] Thus, the perselenenation of Cys_4-type ZPs to $Zn(S\text{-}SeR)_n$ rather than oxidation to the disulfide (ZP_{ox}) is consistent with a lower barrier for selenosulfide formation. For Cys_2His_2-type ZFs, the moderate barrier to the Se−S bond allows monoselenenated ZP(S−SeR) to exist long enough for unimolecular attack of the Cys thiolate upon selenosulfide to form Zn_{ox} ($k_{ox} > k_{per}$; Scheme 2). For Cys_4-type ZFs and MT, selenosulfide bond formation is much faster ($k_{per} > k_{ox}$), such that, in the presence of excess rSe compounds, each Cys reacts with 1 equiv of rSe to release Zn^{2+}. Note that the reactivity of Cys_3His-type ZPs with rSe compounds has been less well-studied, and its moderate nucleophilicity relative to Cys_2His_2 and Cys_4 may lead to other mechanistic pathways.

DFT modeling of selenosulfide formation by the attack of **1** on a ZFM is an important step toward understanding the electronic factors involved in the reactivity of ZFs. The simplified ZFM omits steric effects to provide a baseline for the interaction with **1**. Future studies will explore the reactivity of Cys_3His- and Cys_4-type ZFMs with other rSe compounds known to release Zn^{2+}. These results and their extension into larger models will enhance the design of selenium-based drugs to target ZFs involved in cancer and viral infections as well as increase our understanding of the toxic effects of selenium.

■ ASSOCIATED CONTENT

Supporting Information

Details of DFT calculations and Cartesian coordinates of the structures in Figure 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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