Effect of Methylmercury Binding on the Peroxide-Reducing Potential of Cysteine and Selenocysteine

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ABSTRACT: Methylmercury (CH$_3$Hg$^+$) binding to catalytically fundamental cysteine and selenocysteine of peroxide-reducing enzymes has long been postulated as the origin of its toxicological activity. Only very recently, CH$_3$Hg$^+$ binding to the selenocysteine of thioredoxin reductase has been directly observed [Pickering, I. J. et al. Inorg. Chem., 2020, 59, 2711−2718], but the precise influence of the toxicant on the peroxide-reducing potential of such a residue has never been investigated. In this work, we employ state-of-the-art density functional theory calculations to study the reactivity of molecular models of the free and toxified enzymes. Trends in activation energies are discussed with attention to the biological consequences and are rationalized within the chemically intuitive framework provided by the activation strain model. With respect to the free, protonated amino acids, CH$_3$Hg$^+$ binding promotes oxidation of the S or Se nucleus, suggesting that chalcogenoxide formation might occur in the toxified enzyme, even if the actual rate of peroxide reduction is almost certainly lowered as suggested by comparison with fully deprotonated amino acids models.

1. INTRODUCTION

Physiological thiols and selenols are widely recognized as methylmercury (CH$_3$Hg$^+$) targets.$^{1-4}$ In the biological environment, cysteine (Cys) and selenocysteine (Sec) constitute the main thiol- and selenol-containing compounds and are catalytically fundamental residues in the enzymatic activity of the glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) families, whose inhibition has been demonstrated to be implicated in CH$_3$Hg$^+$ toxicity.$^{2,3,5-7}$ These enzymes, particularly GPx, are peroxide-reducing enzymes, which contribute to keep regulated the peroxide tone of the cell.$^6$ CH$_3$Hg$^+$ possesses pro-oxidative properties, likely via GPx inhibition, which leads to the accumulation of hydroperoxide and to hydroperoxide-mediated excitotoxicity, which have been associated with its neurotoxicity.$^{4,6,9}$ Despite the toxicological knowledge about GPx inhibition by CH$_3$Hg$^+$, the chemical details behind its interaction with thio- and selenoproteins are not known, and precise knowledge about its toxicological mechanism has not been achieved.$^5$ Particularly, only very recently, the binding between the toxicant and the catalytically fundamental Sec of TrxR has been observed directly,$^{10}$ in an elegant study by Pickering et al. They succeeded in detecting the Se-Hg bond by means of extended X-ray absorption fine structure spectroscopy.

The effect of CH$_3$Hg$^+$ binding on the chalcogen nucleus implicated in the catalytic mechanism of such peroxide-reducing enzymes has never been investigated. In fact, sulfur and selenium possess a central role in the antioxidant system of living beings and both endogenous antioxidant molecules and peroxide-reducing enzymes employ S or Se to fulfill their role. Seminal computational investigations on (methyl)mercury chalcogenolate complexes have been previously performed by Schreckenbach et al.$^{11}$ Particularly, they highlighted how the chalcogenophilicity of mercury is the same in systems of increasing complexity,$^{12}$ and they investigated peculiar reactivity aspects of free methylmercury selenocysteinate complexes, which leads to their degradation.$^{13}$ In fact, methylmercury−selenium binding is on the basis of the so-called selenium−mercury antagonism. Hg-containing compounds can cause selenium depletion due to the formation of mercury selenide nanoparticles (HgSe), which could disrupt the synthesis of selenoenzymes and increase Hg neurotoxicity. In contrast, HgSe formation could also antagonize CH$_3$Hg$^+$ toxicity because of the far less toxic properties of such nanoparticles.$^{14-16}$ However, a careful investigation of how CH$_3$Hg$^+$ affects the peroxide-reducing capabilities of Cys and Sec residues is still missing and it is important to understand the fate of the enzyme after CH$_3$Hg$^+$ binding.

It is well known that GPx operates via a three-step mechanism (Scheme 1, left), where the first step is the...
effective peroxide reduction.\textsuperscript{17,18} Since the first step can in principle occur with some changes even after CH\textsubscript{3}Hg\textsuperscript{+} binding (Scheme 1, right), such an event deserves deeper scrutiny, and it is the main topic of this work.

The GPx first step has recently attracted great attention, and it has been demonstrated that for GPx\textsuperscript{18,19} and other important Cys/Sec-based enzymes,\textsuperscript{20} peculiar features of the catalytic pocket enable Cys and Sec to display peroxidatic activity. Particularly, Cys and Sec can become deprotonated through a proton transfer to a nearby acceptor site, leading to a charge-separated intermediate. When (Cys/Sec\textsuperscript{−}) attacks one oxygen of the peroxide bond, the proton is shuttled to the opposite oxygen atom, enabling an efficient cleavage of water from the substrate. Such a mechanism, however, is only possible in suitably designed molecular architectures like a catalytic pocket and requires a proton-acceptor in a specific position, optimal for both deprotonating Cys/Sec and donating the proton to the peroxide bond. Evidently, after complexation by CH\textsubscript{3}Hg\textsuperscript{+}, the formation of a charge-separated intermediate is inhibited, and this might be enough to impair enzyme functionality. However, a thorough investigation of the effect of CH\textsubscript{3}Hg\textsuperscript{+} binding to thio- and selenoproteins based on molecular models is recommended.

\textit{In silico} investigation on the oxidation of molecular organochalcogen compounds is not unusual in the literature,\textsuperscript{21–23} and for both organosulfur and organoselenium compounds, different mechanistic pathways have been investigated.\textsuperscript{24–27} Particularly, focusing on thios, it has been proven that the reaction occurs faster when the system is deprotonated or when deprotonation occurs at the transition state, in a proton shuffling manner.\textsuperscript{26} However, focusing on Cys, Sec, and tellurolcysteine (Tec), the comparative studies are rare. Particularly, Cardy et al.\textsuperscript{27} compared Cys to Sec oxidation by hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) in their deprotonated form, noting an important impact of the conformation on the activation energy, with Sec having a moderately lower barrier with respect to Cys of about 3 kcal mol\textsuperscript{−1}. Thus, they concluded that the Sec peroxidatic behavior is enhanced only when deprotonation occurs at the transition state, in a proton shuffling manner.\textsuperscript{26} However, focusing on Cys instead of Sec, while such a mechanism was not identified for Tec, likely because of the hydride character of the Te–H bond.\textsuperscript{28}

The scope of this work is to understand the influence of CH\textsubscript{3}Hgl\textsuperscript{+} on the chalcogen nucleus oxidation by hydrogen peroxide in model methylmercury (seleno/telluro) cysteinate complexes. While (seleno)cysteine binding with CH\textsubscript{3}Hg\textsuperscript{+} is relevant for toxicological reasons, tellurolcysteine is included for completeness and because tellurocys might have a role in methylmercury detoxification in virtue of their high mercury binding capabilities.\textsuperscript{29,30}

2. COMPUTATIONAL METHODS

All DFT calculations have been performed with the Amsterdam Density Functional (ADF) program\textsuperscript{31} 2018 and 2019 version. Zeroth-order regular approximation (ZORA) has been employed to include relativistic effects in the calculations, as recommended in the presence of heavy atoms.\textsuperscript{32} In all calculations, BLYP\textsuperscript{33,34} functional has been used with the inclusion of Grimme dispersion with the Becke–Johnson damping function.\textsuperscript{35–38} For all atoms, the TZ2P basis set has been used, which is a large uncontracted set of Slater-type orbitals of triple-ζ quality, augmented with two sets of polarization functions per atom. In all calculations, small frozen core approximation has been employed. Such a level of theory is from now on denoted as ZORA–BLYP-D3(BJ)/TZ2P and was previously benchmarked for methylmercury chalcogenolate structures and reactivity.\textsuperscript{39} Moreover, it has been previously applied for the investigation of the oxidation of organochalcogen compounds by H\textsubscript{2}O\textsubscript{2}.\textsuperscript{40} For solvent-assisted proton-exchange (SAPE) calculations, activation energies have been computed also employing B3LYP\textsuperscript{41,42} functional on the BLYP-D3(BJ)\textsuperscript{33} optimized geometries, since hybrid functionals with a low percentage of Hartree-Fock orbitals of triple-ζ quality, augmented with two sets of polarization functions per atom. Inorganic Chemistry pubs.acs.org/IC 4647

**Scheme 1. GPx Catalytic Cycle (Left)**\textsuperscript{44}

The first step (1) is the actual peroxide reduction, displaying the conversion of a selenol (−SeH) to a selenenic acid (−SeOH) that can be reduced back to a selenol by two glutathione (GSH) molecules. (Right) CH\textsubscript{3}Hg\textsuperscript{+}-inhibited Sec GPx mechanism of peroxide reduction, as has been postulated. The step, equivalent to step (1) of the functional GPx, displays the oxidation of a methylmercury selenolate to a selenoxide, with consequent peroxide reduction.

\[ \Delta E(\xi) = \Delta E_{\text{trans}}(\xi) + \Delta E_{\text{int}}(\xi) \] (1)

where \( \Delta E_{\text{trans}}(\xi) \) is the deformation energy required to distort the reactants into the geometry they display at the point \( \xi \) along the rc, while \( \Delta E_{\text{int}}(\xi) \) accounts for the chemical interactions between the distorted reactants. \( \Delta E_{\text{int}}(\xi) \) can be further decomposed into different chemically meaningful terms within the EDA scheme.
ΔE_{\text{elstat}}(\zeta)\text{ accounts for the semiclassical electrostatic interaction between the unperturbed electronic densities of the two approaching fragments; } ΔE_{\text{Pauli}}(\zeta)\text{, namely, Pauli repulsion, the repulsive interaction between occupied orbitals, and } ΔE_{\text{oi}}(\zeta)\text{, the orbital interaction such as highest-occupied molecular orbital (HOMO)−lowest-unoccupied molecular orbital (LUMO) interaction} \text{ and the dispersive interaction } ΔE_{\text{disp}}(\zeta)\text{, which is taken into account at the level of theory of the calculations [i.e., D3(BJ)]. ASA and EDA at single-point geometries have been executed manually as implemented in ADF. Conversely, along the whole rc, they have been performed using IRC geometries with the program PyFrag.}^{55}

Gibbs free energies at 298.15 K and 1 atm have been computed by means of standard statistical-thermodynamics relationships within the ideal gas approximation, employing electronic energies and frequencies. Activation and reaction free energies display the same trends (Tables S5 and S6) and are not discussed in the main text to keep consistency with ASA and EDA that can be performed on electronic energies only.

3. RESULTS AND DISCUSSION

To gain insight into how CH₃Hg⁺ binding affects the peroxide-reducing potential of Cys, Sec, and Tec, we have investigated in silico the mechanistic details of Cys, Sec, and Tec oxidation by H₂O₂ and the analogous mechanisms for methylmercury cysteinate, selenocysteinate, and tellurocysteinate complexes (MeHgCys, MeHgSec, and MeHgTec, respectively). For completeness, reaction and activation energies have been calculated also for Cys⁻ and Sec⁻, which are the two relevant systems from a biochemical point of view of catalysis, to take into account the effect of full deprotonation as it might occur in solution at medium−high pH, and in the active site of peroxidatic enzymes. These systems have been chosen as models of the fully functional and toxified enzymes. However, even such situation does not exactly match to the one occurring inside GPx, where the chalcogenolate attack to H₂O₂ occurs without any appreciable barrier and thus is not rate-determining.²⁸

Three different mechanisms have been investigated for the amino acid oxidation. First, a stepwise mechanism has been followed for the oxidation of the chalcogenols to the corresponding chalcogenenic acids, via a chalcogenoxide intermediate (Scheme 2a). The proton shuttling at the transition state is depicted in parentheses.

\[
ΔE_{\text{tot}}(\zeta) = ΔV_{\text{detal}}(\zeta) + ΔE_{\text{Pauli}}(\zeta) + ΔE_{\text{oi}}(\zeta) + ΔE_{\text{disp}}(\zeta)
\]

where \(ΔV_{\text{detal}}(\zeta)\) accounts for the semiclassical electrostatic interaction between the unperturbed electronic densities of the two approaching fragments; \(ΔE_{\text{Pauli}}(\zeta)\), namely, Pauli repulsion, the repulsive interaction between occupied orbitals, and \(ΔE_{\text{oi}}(\zeta)\), the orbital interaction such as highest-occupied molecular orbital (HOMO)−lowest-unoccupied molecular orbital (LUMO) interaction and the dispersive interaction \(ΔE_{\text{disp}}(\zeta)\), which is taken into account at the level of theory of the calculations [i.e., D3(BJ)]. ASA and EDA at single-point geometries have been executed manually as implemented in ADF. Conversely, along the whole rc, they have been performed using IRC geometries with the program PyFrag.²⁵

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Such a mechanism displays high activation energies and significantly differs from the enzymatic one, especially because of the high isomerization barrier required to pass from the chalcogenoxide to the chalcogenenic acid.²⁵,²⁶ Conversely, the oxidation to telluroxide has been hypothesized for Tec in mechanistic studies in enzyme, and it is likely possible also for the free Tec.²⁸ Even if this pathway is unlikely to be
responsible for Cys/Sec oxidation to the relative acids, it is valuable from a theoretical point of view: in fact, replacing $-\text{H}$ with $-\text{HgCH}_3$, the oxidation to chalcogenoxide can be modeled in an identical way, thus showing us the mere effect of the substituent (Scheme 2b).

To more properly model the oxidation of our systems in the presence of a few water molecules (as they are usually present in the catalytic pockets of enzymes such as GPx), a second pathway has been investigated employing the so-called solvent-assisted proton-exchange (SAPE) approach, which has been extensively applied by Bayse et al. to organochalcogen reactivity. In this mechanism, a proton shuttles through a network of hydrogen-bonded water molecules and $\text{H}_2\text{O}$ and leads to peroxide reduction in a concerted manner. We employed two water molecules in our SAPE network (Scheme 3), because only a couple of them are usually present inside the catalytic pocket of GPx.

Finally, the direct oxidation of Cys$^-$ and Sec$^-$ has been followed along a concerted mechanism analogous to that previously reported for these amino acids and for simpler chalcogenolates. This pathway, which leads to water and to chalcogenoxide, will be called anionic mechanism (Scheme 4).

The GPx-like, stepwise mechanism via a charge-separated intermediate has not been investigated because it would have required the definition of an enzyme-specific proton-acceptor as it has been recently done in a work by some of us, and it was shown to display unfeasible activation energies when the whole enzyme environment is not involved. It is possible, however, that the introduction of a proton acceptor strategically placed near the substrate/hydrogen peroxide might promote a proton shuffling mechanism, thus further lowering the activation energy of the SAPE mechanism.

Cys, Sec, and Tec have been optimized starting from the minimum-energy conformer reported in the literature for Cys. For the relative complexes with methylmercury, $-\text{H}$ has been replaced with $-\text{HgCH}_3$ and a full optimization was carried out, while for the anionic structures, we reoptimized after $\text{H}^+$ removal. The oxidation to chalcogenoxide can proceed along two diastereoismeric pathways, due to Cys/Sec/Tec stereogenic carbon atom and to the chalcogen nucleus at which the oxidation occurs. No great energetic or mechanistic differences have emerged along the two pathways for organoseleno oxidation. Thus, only one pathway has been fully investigated. With the aim of understanding the CH$_2$Hg$^+$ effect, we have followed the path along which the reaction energies with and without CH$_2$Hg$^+$ showed slightly greater differences. Thus, we have not followed the path going through the lowest-energy diastereoisomer, but rather the one through the least stable as it followed from preliminary energetic analysis on methylselenocysteine (MeSec) and methylmercury selenocysteinate (MeHgSec). For the protonated systems and methylmercury complexes, we followed the oxidation of the (R, R) diastereoisomer, for which both the stereogenic carbon of the amino acids and the stereogenic chalcogen atom have the R absolute configuration. For the anionic mechanism, we ensured to follow the reaction pathway leading to structurally analogous products.

3.1. Mechanistic Details. The stepwise pathway (Scheme 2) has been found for all three residues, and the oxidative step (Scheme 2, step 1) has been found also when replacing $-\text{H}$ with $-\text{HgCH}_3$ (Scheme 2b). In all cases, the reaction goes through similar structures. First, a reactant complex (RCox) is formed, where hydrogen peroxide is located in proximity of the chalcogen nucleus. The oxidation takes place crossing a transition state (TSox), where the O−O bond of hydrogen peroxide is breaking apart, while the O−X (X = S, Se, Te) bond is forming. The reaction leads to a weakly bonded product complex (PCox) with a water molecule coordinated to the chalcogenoxide group. The oxidized product (Pox) is the same species after removal of the water molecule (i.e., the water molecule and the chalcogenoxides are infinitely distant and are thus considered free products). Upon oxidation, the conformation of the amino acids slightly changes, due to the interaction between the aminic function and the chalcogen oxide moiety, as previously observed for methylselenocysteine. Analogous structures were located for the corresponding methylmercury complexes, with a few minor differences: in the reactant complexes, $\text{H}_2\text{O}$ is also in proximity of Hg nucleus, and in the oxidized products, the slightly rotated conformation has not been located because the complex retains the same conformation of the reactant after oxidation. For the free amino acids, the isomerization occurs crossing a transition state (TSiso) that connects the chalcogenoxide Pox to the chalcogenic acid Piso (Figure 1). For the complexes, the isomerization pathway to the chalcogenic acid is not possible; thus, only the oxidation to chalcogenoxide has been investigated, even if further evolution of the chalcogenoxide cannot be ruled out. The oxidative step of the stepwise mechanism for free amino acids and the oxidation to chalcogenoxide for the complexes will be from now on referred to as the minimal mechanism.

All of the reactions are characterized by a high-energy transition state for the isomerization process, as previously reported (Figure S1), with the activation energy required for...
the oxidative step that decreases when going from Cys to Sec and to Tec, in agreement with the enhanced peroxidatic activity of organoseleno and organotelluro compounds with respect to the analogous organosulfur compounds (Table 1).24,41

For Cys and Sec only, the transition states along the SAPE mechanism have been located. All of the attempts to find the analogous TS for Tec failed, suggesting similarity of Tec behavior inside the enzyme and in the free form.28 Similar structures were located for Cys and Sec, with a TS state connecting an RC to a PC. At the TS, the X–H (X = S, Se) bond is elongated, indicating partial deprotonation and proton shuttling through a hydrogen-bond network involving Cys, H2O2, and two water molecules, while the peroxide O–O bond is breaking apart. For methylmercury complexes, similar structures were located. In this case, however, the reaction evolves toward the chalcogenoxides and the complex does not participate in the hydrogen-bond network, which involves only the two water molecules and H2O2 (Figure 2).

The SAPE mechanism reduces the activation energy required for the oxidation of both free amino acids and the methylmercury complexes (Table 2 vs Table 1), leading to more feasible barriers, as it was previously described for Cys, for which SAPE proved to be a valuable mechanism to reproducing experimentally detected activation energies.25

For what concerns the anionic mechanism for Cys and Sec, oxidation proceeds in a single step from chalcogenolates to deprotonated chalcogenenic acids, with a transition state connecting a reactant complex to a product complex that closely resembles those of the minimal pathway. In this case, in the RC, hydrogen peroxide assumes a trans-like conformation as shown in Figure 1; however, it is significantly closer to the amine function. Moreover, with respect to the minimal mechanism, in the TS, hydrogen peroxide is far less distorted. Such mechanism displays the lowest activation energy of all of

| R   | RCox | TSox   | PCox | PoX |
|-----|------|--------|------|-----|
| Cys | 0.00 | −6.01  | 17.84(23.85) | −47.88 | −38.84 |
| Sec | 0.00 | −6.02  | 14.06(20.08) | −40.04 | −29.64 |
| Tec | 0.00 | −6.04  | 6.25(12.29)  | −48.51 | −37.72 |
| Cys’| 0.00 | −19.86 | −13.01(6.85) | −65.14 | −50.17 |
| Sec’| 0.00 | −18.7  | −13.51(5.19) | −59.92 | −44.08 |
| MeHgCys | 0.00 | −8.61  | 12.79(21.40) | −47.09 | −33.81 |
| MeHgSec | 0.00 | −8.44  | 9.76(18.20)  | −39.38 | −24.33 |
| MeHgTec | 0.00 | −7.82  | 3.89(11.71)  | −44.37 | −28.68 |

“For Cys, Sec, Tec, MeHgCys, MeHgSec, and MeHgTec, the reaction evolves to the corresponding chalcogenoxides, while for Cys” and Sec”, to deprotonated chalcogenenic acids. Activation energies relative to the RC are given in parentheses. Level of theory: ZORA–BLYP-D3(BJ)/TZ2P. $^b$RC for MeHgTec converged in a slightly different conformation for H2O2 with respect to MeHgCys and MeHgSec.

| R   | RC  | TS   | PC  | P   |
|-----|-----|------|-----|-----|
| Cys | 0.00| −22.92| −12.65(10.27) | −80.81 | −49.83 |
| Sec | 0.00| −22.43| −15.67(6.77)  | −84.88 | −55.42 |
| MeHgCys | 0.00| −23.86| −16.54(7.33)  | −67.25 | −33.81 |
| MeHgSec | 0.00| −23.29| −19.73(3.65)  | −61.09 | −24.33 |

“For Cys and Sec, the reaction evolves to the corresponding chalcogenenic acids, while for MeHgCys and MeHgSec, to the chalcogenoxides. Activation energies relative to the RC are given in parentheses. Level of theory: ZORA–BLYP-D3(BJ)/TZ2P.
the three under investigation for Cys and Sec oxidation, in agreement with the enhanced nucleophilicity of deprotonated chalcogenols.

3.2. Methylmercury Effect. Analogous pathways have been investigated for both free and complexed amino acids, and so information about the influence of CH$_3$Hg$^+$ binding on the chalcogen nucleus oxidation can be obtained. The results are reported in Table 1 for the minimal and anionic mechanism, and in Table 2 for the SAPE mechanism.

The replacement of $-\text{H}$ with $-\text{HgCH}_3$ leads to a systematic decrease of the activation energy required for the oxidation. For Cys and Sec, which are the biologically relevant systems, CH$_3$Hg$^+$ binding leads to a moderate but appreciable decrease in activation energy of about 2—3 kcal mol$^{-1}$ in both mechanisms, which is comparable to the rather modest substituent effect for dichalcogenides oxidations studied by some of us.41 Methylmercury chalcogenolates display slightly less negative reaction energies (i.e., less favored reactions). This is likely because the complexes retain their conformation after oxidation, while the free amino acids slightly rearrange. This effect is more prominent along the SAPE pathway, because in this case, the products themselves already show a different stability, with the acids being at a more negative energy with respect to the corresponding oxides. Thus, this is the determinant factor decreasing energetic feasibility (Figure S1). However, all reactions display a prominent negative reaction energy (Figure 3).

The comparison of the anionic pathway (Cys$^-$ and Sec$^-$) and the minimal pathway (MeHgCys and MeHgSec) shows an opposite trend (Table 1 and Figure 3). With respect to fully deprotonated amino acids, CH$_3$Hg$^+$ binding significantly increases the activation energy required for peroxide reduction, as it is expected because the chalcogenolates lose the negative charge that promotes an $\text{S}_\text{N}2$-like reaction. Thus, methylmercury chalcogenolate complexes are predicted to reduce hydrogen peroxide at a lower rate than the fully deprotonated amino acids, but anyway faster than the respective protonated amino acids.

3.3. Activation Strain Analysis and Methylmercury Effect. The activation strain model of chemical reactivity (or activation strain analysis, ASA) has been employed as described in the computational methods to gain insight into
transition state stabilization of the methylmercury complexes with respect to the protonated amino acids. The same method has been successfully applied in precedent studies on dichalcogenides \(^{41}\) and chalcogenols. \(^{24}\) First, the minimal mechanism will be discussed, since similar conclusions can be drawn for all of the systems.

ASA along the whole reaction coordinate, using IRC geometries, has been carried out for the minimal mechanism of \((\text{MeHg})\text{Cys}\), \((\text{MeHg})\text{Sec}\) (Figure 4), and \((\text{MeHg})\text{Tec}\) (Figure S2). The system has been partitioned into two fragments, i.e., the chalcogenol/complex and the peroxide, to relate the trends in reactivity to the reactant properties. Then, for both the minimal and the SAPE models, we performed ASA and EDA at the RC and TS.

It is straightforward to assess that methylmercury chalcogenolates undergo faster oxidation mainly because of a larger stabilizing interaction energy. Even if for MeHgCys and MeHgSec the transition state occurs slightly later along the rc, this does not affect significantly the reactivity of these systems, since the strain profiles are almost completely superimposed to those of Cys and Sec. The rather limited influence of replacing \(-\text{H}+\) with \(-\text{HgCH}_3\) on \(\Delta E_{\text{strain}}\) was expected, since for this kind of reaction, most of the strain is due to \(\text{H}_2\text{O}_2\) deformation, which undergoes the same structural modifications when changing the substrates. Thus, further analyses have been done as single-point ASA and EDA at the TS and RC.

From EDA (Tables S7 and S8), it emerges that the larger stabilizing \(\Delta E_{\text{int}}\) for the complexes is due to the interplay between \(\Delta E_{\text{Pauli}}\), \(\Delta E_{\text{oi}}\), and \(\Delta V_{\text{elstat}}\). Changing the substituent from \(-\text{H}\) to \(-\text{HgCH}_3\) leads to a decrease in Pauli repulsion (which becomes less destabilizing) and to a decrease (in absolute value) in orbital interaction and electrostatic interaction (that becomes less stabilizing). In the end, the less stabilizing \(\Delta E_{\text{oi}}\) is overcome by the less destabilizing \(\Delta E_{\text{Pauli}}\) which leads to a more stabilizing \(\Delta E_{\text{int}}\) and thus to a lower-energy TS for MeHgX with respect to X.

As it has been extensively investigated for chalcogenols and dichalcogenides, the activation energy of these systems with hydrogen peroxide correlates with the energy of Cys/Sec HOMO. \(^{24,41}\) MeHg(Cys/Sec) displays a higher-energy HOMO, which leads to a less favored energy match to \(\text{H}_2\text{O}_2\) LUMO responsible for the less stabilizing \(\Delta E_{\text{oi}}\) since at the transition state, the LUMO of \(\text{H}_2\text{O}_2\) has a lower energy with respect to the HOMO of the substrate \(^{62}\) (Figure S3). However, even if \(\Delta E_{\text{oi}}\) becomes less stabilizing, as a rule of thumb, the higher the HOMO, the faster the reactivity with \(\text{H}_2\text{O}_2\). Similar conclusions can be drawn when comparing MeHgTec to Tec oxidation (Table S9) and is thus a general effect of \(\text{CH}_3\text{Hg}^+\) binding, which destabilizes Cys/Sec/Tec HOMOs and lowers \(\Delta E_{\text{Pauli}}\).

Along the SAPE mechanism, discrepancies arise both from the exchange of \(-\text{H}\) with \(-\text{HgCH}_3\) and from mechanistic differences that lead to different products (chalcogenenic acids and chalcogenoxides, respectively). Thus, from our ASA, a different picture can be seen with respect to the minimal model, both for Cys (Figure 5) and for Sec (Figure 6).

Moreover, while the definition of the fragments for the minimal model is straightforward, for the SAPE mechanism, it is less trivial. Particularly, to avoid uncommon negative strain energies, we partitioned the system into the chalcogenol/complex and a fragment composed of two water molecules and hydrogen peroxide. We set as a reference point for relative energies the chalcogenol/complex and a fictitious reactant formed after optimization of a ring composed of two water molecules and hydrogen peroxide only (Table S1, fictitious reactant). The trends are consistent with those obtained using as a reference point the free reactants (Table S10), and discrepancies in the energies of RCs and TSs with respect to Table 2 arise from the different choice of the reference state.

For the SAPE oxidation, it is immediately possible to note how MeHgCys undergoes faster oxidation with respect to Cys because of an important lowering of \(\Delta E_{\text{strain}}\) from RC to TS,
despite a less stabilizing $\Delta E_{\text{int}}$. This effect has been attributed to the mechanistic differences between the two reactions, with Cys displaying a higher $\Delta E_{\text{strain}}$ because of $S=H$ bond deformation, which is breaking apart at the transition state. Conversely, MeHgCys does not undergo any major distortion during the reaction, because it is not involved in the SAPE hydrogen-bond network, and this reflects into the lower activation strain required for the process to occur. The difference in $\Delta E_{\text{int}}$ with respect to the minimal mechanism can be rationalized in terms of HOMO–LUMO interaction. MeHgCys still displays a higher HOMO than Cys, as investigated for the minimal mechanism. However, because of the different hydrogen-bond network in Cys and MeHgCys transition states, $H_2O_2$ LUMO for Cys oxidation is significantly higher in energy than in MeHgCys oxidation. In the former case, this reduces the HOMO–LUMO energy gap enough to make $\Delta E_{\text{int}}$ much more stabilizing for Cys than for MeHgCys. Thus, along the SAPE mechanism, the lowering in $\Delta E_{\text{int}}$ that occurs replacing $-H$ with $-HgCH_3$ is not enough to compensate for the loss in $\Delta E_{\text{int}}$ leading to a less stabilizing interaction energy for MeHgCys.

An opposite scenario can be seen for Sec and MeHgSec SAPE oxidation. In this case, MeHgSec displays a more stable TS because of a more stabilizing interaction energy and despite a more destabilizing strain energy (Figure 6).

In this case, this effect is ascribed to the different position along the rc for the two TSs. In fact, while for Cys and MeHgCys, the two transition states occur in close proximity along the rc (with differences on O–O bond of 0.03 Å, and MeHgCys displaying a slightly later transition state similarly to the minimal mechanism), Sec displays a significantly earlier transition state with respect to MeHgSec (with $d_{O-O}$ of 1.77 and 1.91 Å, respectively). Thus, the higher activation strain required to reach MeHgSec TS follows naturally from the more severe deformation of $H_2O_2$ when the more negative $\Delta E_{\text{int}}$ is at least partly a consequence of the closer distance between the two interacting fragments, which in the end leads to a lower-energy TS. Comparing Figure 6 with Figure 5, it is possible to note that with respect to Cys, Sec displays a far less stabilizing $\Delta E_{\text{int}}$, while MeHgCys and MeHgSec show close values of $\Delta E_{\text{int}}$, thus enhancing our confidence in linking the trend differences between the two amino acids and complexes to the relative position along the rc at which their TSs occur.

Finally, ASA (Table 3) and EDA (Table S11) have been performed on the anionic system ($Cys^−$ and $Sec^−$) also, and the results have been compared to the minimal oxidation of MeHgCys and MeHgSec. Along the anionic pathway, the transition state is reached far earlier along the rc, with $d_{O-O}$ around 1.70 Å, while along the minimal pathway, $d_{O-O}$ is around 2.00 Å in both cases.

This affects mainly the strain energy, which is much lower when going from RC to TS along the anionic pathway with respect to the minimal pathway, where stronger interaction energies can be seen. This effect is well documented in the literature and is rooted in the shape of the interaction energy curve. In fact, stronger nucleophiles (i.e., negatively charged in our case) display stronger interaction energies along the whole rc, even if at the TS alone, the analysis can misleadingly suggest otherwise, as it is in our system, because of the different point along the rc at which the analysis has been performed. In fact, for the minimal mechanism (Figure 3, left) at rc = 0.21 Å corresponding to the TS of $Cys^−$, $\Delta E_{\text{int}}$ of MeHgCys is computed to be around $-4.00 \text{ kcal mol}^{-1}$, far less stabilizing than that of $Cys^−$. Thus, we conclude that methylmercury chalcogenolates display later TSs with a higher energy with respect to free chalcogenolates because of a shallower interaction energy curve, in line with the Hammond postulate.

### Table 3. ASA (kcal mol$^{-1}$) for $Cys^−$ and $Sec^−$ and for MeHgCys and MeHgSec$^a$

|            | $\Delta \tilde{E}$ | $\Delta E_{\text{strain}}$ | $\Delta E_{\text{int}}$ |
|------------|---------------------|-----------------------------|--------------------------|
| $Cys^−$    | RC                  | $-19.86$                    | $3.25$                   | $-23.11$ |
|            | TS                  | $-13.01$                    | $9.52$                   | $-22.53$ |
| $Sec^−$    | RC                  | $-18.70$                    | $2.74$                   | $-21.44$ |
|            | TS                  | $-13.51$                    | $8.45$                   | $-21.96$ |
| MeHgCys    | RC                  | $-8.61$                     | $0.29$                   | $-8.90$  |
|            | TS                  | $12.79$                     | $42.84$                  | $-30.05$ |
| MeHgSec    | RC                  | $-8.44$                     | $0.31$                   | $-8.75$  |
|            | TS                  | $7.96$                      | $42.63$                  | $-32.87$ |

$^a$Level of theory: ZORA–BLYP-D3(BJ)/TZ2P.
selenoxide elimination, which has been hypothesized to be responsible for the irreversible inactivation of GPx under highly oxidizing conditions, and it is involved in the irreversible inactivation of small-molecule-inhibited TrxR. Alternatively, further evolution of the selenoxide might lead to the insertion of the oxygen atom between the chalcogen and mercury atom, leading to a structure similar to the one hypothesized for PhSeZnCl oxidation. Also, in that case, the presence of zinc increased the catalytic activity of diphenyl diselenide toward thiol oxidation by hydrogen peroxide. Our study prompts a more detailed investigation of methylmercury chalcogenolates reactivity. Experimental investigation of our trends, both at the single residue and at the enzymatic level, might help reach a better understanding of the evolution of CH₃Hg⁺ toxicity and of the chemical details behind selenoproteins inhibition by methylmercury.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.inorgchem.0c03619.

Cartesian coordinates, electronic energies, and imaginary frequencies of the investigated compounds; Gibbs free energies and energies in water; and additional ASA and EDA calculations (PDF)

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Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was funded by Università degli Studi di Padova, thanks to the P-DiSC (BIRD2018-UNIPD) project MAD5 (Modeling Antioxidant Drugs: Design and Development of computer-aided molecular Systems; P.I.: L.O.). All of the calculations were carried out on Galileo (CINECA: Casalecchio di Reno, Italy) thanks to the ISCRA Grant MEMES (METHylMERCury and Selenoproteins) and MEMES2. L.O. contributed to this research as part of the scientific activity of the international multidisciplinary network “SeS Redox and Catalysis”. J.B.T.R. and P.A.N. thank the financial support by Coordination for Improvement of Higher Education Personnel (no. 23038.004173/2019-93; no. 0493/2019; no. 88882.182123/2018-01) and the Institutional Internationalization Project (CAPES/PrInt) (no. 88887.374997/2019-00).

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