20
Quantum Mechanical Approaches to Selenium Biochemistry

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20.1.
Introduction

Understanding the intricate details of enzyme function at the molecular level is of fundamental importance to biological science. An impressive array of experimental techniques have led to many advances in biochemistry, but often critical information about the energetics and structural features of intermediates and transition states in enzymatic reactions is inaccessible. A major strength of quantum chemical calculations is their ability to elucidate many details of a given molecular system, which serves as a model for the real system. Rapid advances in computer technology as well as in computational algorithms and methods have made computational chemistry a viable and complementary partner to experiment in both chemistry and biochemistry.

Selenium was first discovered by the Swedish chemist Berzelius in 1818 [1] and was named after the Greek goddess of the moon, Selene. Eighteen years later, Lowig prepared diethyl selenide (for information on the nomenclature of selenium compounds, the reader is referred to the work by Guenther [2]), and in doing so became the first person to synthesize a selenium-containing compound [3]. Selenium has a wide variety of applications, particularly in medicine, despite being considered a deadly poison until 1957 when it was found that it was a micronutrient for such organisms as bacteria, birds and even mammals [4]. In addition to medicine, modern advances in selenium chemistry are driven by applications in organic synthesis [5–7], biochemistry [8], ligand chemistry [9] and as precursors for metal organic chemical vapor deposition (MOCVD) of semiconducting materials [10].

The study of the biochemical properties of selenium began in 1973 when it was discovered that it played a vital role as the main component in the active site of the antioxidant enzyme glutathione peroxidase (GPx) [11, 12]. Selenium has since been identified as the main component of the active site in several other enyzmatic systems such as the iodothyronine deiodinases [13–16], thioredoxin reductases [17–22], selenophosphate synthetase [21] and selenoprotein P [23]. The selenium-containing residue in GPx was shown to be selenocysteine (Sec) [24], which is the
selenol (Se-H) analog of the thiol (S-H) amino acid cysteine (Cys). Sec is often referred to as the 21st naturally occurring amino acid because it is produced naturally in the body but, surprisingly, it is not typically included among the 20 amino acids in standard biochemistry texts.

Selenium and sulfur show marked differences in their biological behavior and indeed the substitution of sulfur for selenium in GPx is imperative as it provides the enzyme with its antioxidant ability. Although selenium shares many chemical properties with its group 16 neighbor, the incorporation of a chemically more active selenol moiety into the active site as a substitution for a thiol allows for a dramatic catalytic advantage. Direct comparison of the activity of natural GPx and the mutant murine GPx in which sulfur replaces selenium shows that the sulfur analog is 1000-fold less active than the parent enzyme [25]. Moreover, small molecule organo-selenium antioxidants have inactive sulfur-containing counterparts [26], reinforcing the central role played by selenium and the potential for harnessing its reductive ability.

To this end there have been many experimental and theoretical studies on the potential of synthetic organo-selenium compounds as antioxidants, enzyme inhibitors, antitumor and anti-infective agents, and immunomodulators [27]. The remainder of this chapter will focus on reviewing computational studies of selenium-containing systems, including GPx and several small-molecule GPx mimics with various potential biologic applications. A comprehensive survey of the relevant literature on the quantum biochemistry of organo-selenium compounds is beyond the scope of this chapter. We begin, instead, by briefly reviewing several benchmark studies designed to test quantum mechanical methods for predicting properties of selenium-containing compounds and subsequently summarize several applications of such methods to selenium biochemistry.

20.2 Quantum Mechanical Methods for the Treatment of Selenium

The earliest theoretical work on systems involving selenium focused mainly on the properties of small inorganic compounds, while investigations of the biologic aspects of selenium chemistry appeared later. One of the first papers highlighting calculations on selenium appeared in 1981 with Hinchliffe calculating the electronic structure and properties of OSeCl [28]. Subsequent work included investigation of the selenium–sulfur bond by Laitinen et al. [29], the structures of SeH₂ and SeH₂⁺ using non-relativistic effective core potentials (ECPs) by Muller et al. [30], supplementary d-orbital exponents for hypervalent selenium compounds by Angyan et al. [31], ionization energies of SeH and SeH₂ using a multiconfigurational approach with relativistic ECPs by Balasubramanian et al. [32, 33], and the ionization energies and bond dissociation energies of the SeHₙ series by Binning and Curtiss [34, 35].

The first systematic investigation of quantum mechanical methods appropriate for biologically relevant organo-selenium compounds was reported by the current
20.3 Applications to Selenium Biochemistry

20.3.1 Computational Studies of GPx

Aerobic organisms derive their energy from the reduction of O$_2$ and are therefore susceptible to the damaging effects of small amounts of O$_2^-^\cdot$,*OH and H$_2$O$_2$ that inevitably form during the metabolic consumption of oxygen. These three species, together with unstable intermediates in the peroxidation of lipids, are referred to as reactive oxygen species (ROS) [44] and their adverse effects include, but are not limited to, the destruction of key biologic components and the damage of cell membranes. This condition is referred to as "oxidative stress" [45, 46] and is particularly prevalent in the electron-transfer system of mitochondria [27]. Many conditions such as Alzheimer's disease, myocardial infarction, atherosclerosis, Parkinson's disease, autoimmune diseases, radiation injury, emphysema and sunburn are linked to damage from ROS as a result of an imbalance between
radical-generating and radical-scavenging systems and thus a major research effort is focused on combating oxidative stress.

The defense mechanism of the human body against oxidative stress is elaborate but the key steps involve the dismutation of superoxide (O$_2$•⁻) to H$_2$O$_2$ and O$_3$ by superoxide dismutase (SOD) [47] and the reduction of H$_2$O$_2$ by GPx [44, 48]. GPx reduces hydroperoxides to water (or the corresponding alcohol in the case of organic hydroperoxides) at the expense of a glutathione (GSH) cofactor [49] via the mechanism shown in Scheme 20.1.

![Scheme 20.1 Catalytic cycle of GPx.](image)

The selenium moiety in the Sec residue of the enzyme (enzyme-Se-H) is oxidized to the selenenic acid derivative (enzyme-Se-OH) by reduction of peroxide to the corresponding alcohol in step (i). The thiol GSH will then attack the selenenic acid and displace a water molecule to form a seleno-sulfide (enzyme-Se-S-G) intermediate in step (ii). To complete the cycle, a second GSH converts the seleno-sulfide intermediate back into the original selenol by liberating the oxidized glutathione (GSSG) in step (iii). Thus, two GSH molecules are consumed in the process, generating GSSG.

There are four families of GPx enzymes in mammalian organisms [25, 48, 50, 51], including the classical cytosolic GPx (cGPx), which are found in the cytosolic and mitochondrial compartments; phospholipid hydroperoxide GPx (PHGPx), which are intracellular and partially membrane-bound; plasma GPx (pGPx), which are plasmaspecific enzymes; and gastrointestinal GPx (gGPx), which resides in the gastrointestinal tract. Wherever possible, the specific acronyms will be used in the text though collectively they shall be referred to as simply GPx.

The crystal structure of human pGPx was solved in 1997 and at that time it was discovered that the enzyme is tetrameric, consisting of two asymmetric units, each made up of a dimer with half-site reactivity [52]. Comparison of the crystal structure of human pGPx with that of bovine erythrocyte cGPx [24] reveals that in the latter there are two water molecules present in the active site that cannot be observed in the former due to the resolution of the crystal data. A key question is whether water is present in the active site of human pGPx and what role it plays in the antioxidant activity of the enzyme.

Morokuma et al. have pursued this question theoretically using both density functional theory and the hybrid quantum mechanics/molecular mechanics (QM/MM) method [53]. The structure of the active site of human pGPx was modeled using DFT at the B3LYP/6-31G(d) level with and without water molecules present. In
addition, the entire enzyme (monomer) was modeled using several variations of the QM/MM scheme where the active site was modeled using quantum mechanics [in this case HF/STO-3G or B3LYP/6-31G(d)] and the remainder of the structure is treated with molecular mechanics [in this case Amber]. The results show that the root-mean-square deviation between the calculated structure and the crystal structure is minimized when two water molecules are included in the active site, thus providing strong evidence for the presence of two bound water molecules in the active site of human pGPx, especially in light of the fact that two water molecules are observed in bovine cGPx.

The peroxidase activity of pGPx has also been investigated theoretically using the same methods [54, 55]. The full catalytic cycle (as illustrated in Scheme 20.1) of pGPx has been modeled using an "active site only" system with the B3LYP/6-31G(d) method and using the full enzyme (monomer) with the QM/MM method employing B3LYP/6-31G(d)/Amber. Some important conclusions drawn from the study include the effect of residues neighboring the Sec throughout the course of the cycle. In particular Ctn83, Gly50 and the two water molecules provide stabilizing interactions and the predicted overall barrier for the formation of the selenenic acid (enzyme-Se-OH) is 18.0 kcal mol\(^{-1}\) (1 kcal = 4.184 kJ), which is in good agreement with the experimental value of 14.9 kcal mol\(^{-1}\) [56]. Experimentally, the Ctn83 residue has been suspected of participating in the antioxidant activity of GPx [24] and these calculations reveal that it plays a major role by facilitating proton transfer and providing an H-bond acceptor throughout the catalytic cycle. Proton transfer is also shown to be facilitated by the active site's water molecules, elucidating the role water plays in the catalytic activity of pGPx. The effect of the rest of the surrounding protein is predicted to be minimal, generating an increase in the overall barrier of the reaction of only 0.70 kcal mol\(^{-1}\); however, this is probably because the active site is located on the surface of the enzyme.

20.3.2 Computational Studies on GPx Mimics

20.3.2.1 GPx-like Activity of Ebselen
An increase in GPx activity results in an increased ability to cope with oxidative stress. This has been observed in endothelial cells injected with purified GPx, which show a marked increase in survival on exposure to hyperoxia and redox cyclers [57–59]. At first glance, this provides a promising defensive agent against the many detriments of oxidative stress and one might conclude that a wide variety of conditions may be treated with purified GPx. However, natural GPx proteins are not likely to be utilized as pharmacologic agents to increase intracellular GPx activity in vivo because they cannot be expressed in prokaryotes and they are not compatible with oral administration and cellular targeting [60]. For this reason it is desirable to develop synthetic GPx mimics, and indeed many organo-selenium compounds have been studied as bio-models that simulate catalytic functions of various enzymes, including GPx [27].

These model systems have a wide range of basic structures; however, none has received more attention than ebselen [2-phenyl-1,2-benziselenazol-3(2H)-one] (1).
Ebselen is a cyclic selenamide that has been extensively studied as an antioxidant and GPx mimic [61–66]. Although there have been many proposed GPx mimics, ebselen was the first compound to be used in clinical trials [61–63] and has attracted interest because of its anti-inflammatory, antiatherosclerotic and cytoprotective properties in both in vitro and in vivo models [67–71].

Ebselen was first prepared in 1924 by Lesser and Weiss [72]; however, it took six decades to realize its potential as a GPx mimic [61] by reacting slowly with H₂O₂ and other peroxides to afford a stable selenoxide product (6). Although ebselen lacks a selenol moiety (Se–H) – and, therefore, also lacks structural similarity to the active site Sec residue in any selenoenzyme – the selenamide bond is readily cleaved by thiol (Scheme 20.2) to afford a selenosulfide (2), which can then be converted into the selenol derivative of ebselen (3) by an additional thiol. In fact, a diselenide (7) can also be formed via the combination of the original ebselen molecule and the selenol derivative [73]. Scheme 20.2 illustrates the available pathways for interconversion of ebselen (1) to its diselenide (7) and selenol (2) derivatives and for the reduction of peroxides by each [73–75].

The selenol form of ebselen, or the selenolate anion (Se⁻) to be more precise, is the active form in terms of the reduction of hydrogen peroxide and indeed it would seem

Scheme 20.2 Summary of catalytic cycles involving the reduction of peroxides by ebselen, ebselen selenol and ebselen diselenide.
that the existence of a selenol functionality would be required for a GPx-like mechanism (i.e., 3 → 4 → 2). Nevertheless, GPx mimics in the literature have a wide range of structures, including selenamides [76–79], diselenides [74, 80–84], allyl selenides [74], aryl selenides [85] and seleninate esters [86]. Although there are key differences, many of these compounds are related to one of the three derivatives of ebselen in the redox map in Scheme 20.2; that is, these mimics are usually selenols, diselenides or have a divalent selenium atom bound within a heterocycle. Consequently, the reductive pathway of each becomes an important piece of the puzzle in understanding selenium bioactivity, not just that of the selenol.

The catalytic cycle of ebselen has been controversial as a consequence of major differences in working conditions such as solvents, pH and the choice of peroxide. For this reason, several mechanisms have been proposed; however, the most reliable data under physiologically relevant conditions is that shown in Scheme 20.2. To better understand the role played by ebselen, ebselen diselenide, ebselen selenol and the selenolate anion, the current authors carried out two concurrent DFT studies of the reduction of hydrogen peroxide, the first using model compounds [87] and the second using the full structures [88]. The model compounds were constructed to be the simplest structures that maintained the immediate bonding environment about the selenium atom (Scheme 20.3).

![Chemical structure](image)

**Scheme 20.3** Model reactions for the reduction of hydrogen peroxide by ebselen (9), ebselen selenol (11) and ebselen diselenide (13).

For this investigation geometry optimizations were performed with Becke’s three-parameter exchange functional (B3) in conjunction with the correlation functional proposed by Perdew and Wang (PW91) using a 6-311G(2df,p) basis set as suggested previously for the reliable prediction of organo-selenium geometries...
Table 20.1 summarizes the reaction Gibbs energy barriers. The barriers are largely overestimated as Moregenstern et al. [94] have determined the experimental Gibbs energy barriers for ebselen, ebselen diselenide and ebselen selenol to be 18.6 ± 0.2, 18.1 ± 0.1 and 16.5 ± 0.1 kcal mol⁻¹, respectively. This overestimation is predominantly due to the model compounds themselves, as can be demonstrated from the complexes predicted in the oxidation of the ebselen model (Figure 20.1).

Table 20.1. Summary of Gibbs energy barriers for the reduction of hydrogen peroxide by models of ebselen, ebselen selenol and ebselen diselenide (Scheme 20.3).

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Gibbs energy barrier (kcal mol⁻¹)</th>
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<tbody>
<tr>
<td>Ebselen model oxidation (step 1; 9 → 10)</td>
<td>56.7</td>
</tr>
<tr>
<td>Ebselen model oxidation (step 2; 9 → 10)</td>
<td>6.4</td>
</tr>
<tr>
<td>Solvated ebselen model oxidation (step 1; 9 → 10)</td>
<td>60.2</td>
</tr>
<tr>
<td>Solvated ebselen model oxidation (step 2; 9 → 10)</td>
<td>8.5</td>
</tr>
<tr>
<td>Ebselen selenol model oxidation (13 → 14)</td>
<td>53.4</td>
</tr>
<tr>
<td>Solvated ebselen selenol oxidation (13 → 14)</td>
<td>57.7</td>
</tr>
<tr>
<td>Ebselen selenol model anion oxidation</td>
<td>49.4</td>
</tr>
<tr>
<td>Ebselen diselenide oxidation (13 → 14)</td>
<td>35.3</td>
</tr>
<tr>
<td>Solvated ebselen diselenide oxidation (13 → 14)</td>
<td>29.6</td>
</tr>
</tbody>
</table>

and energetics [36]. Transition states were located using Schlegel's synchronous transit-guided quasi-Newton (STQN) method [89, 90] and were linked to reactant and product complex structures by the use of an intrinsic reaction coordinate calculation [91, 92]. Frequency calculations were performed on all optimized structures using the B3PW91/6-311G(2df,2p) method to obtain accurate thermochemical data and to confirm whether a structure was a minimum or first-order saddle point; accurate energies were obtained for all structures via single-point calculations using the 6-311++G(3df,3pd) Pople basis set with the above DFT method. Solvent effects were incorporated implicitly with single-point calculations using the conductor-like polarizable continuum model (CPCM with a dielectric constant of 78.39 for water) at the B3PW91/6-311++G(3df,3pd) level and explicitly (for the case of the deprotonated selenol anion reaction) with the inclusion of three water molecules. For the selenol anion reaction, diffuse functions were also included on heavy atoms for the geometry optimizations and frequency calculations as well as in the transition state searches.

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The reaction coordinate of this species illustrates a two-step conversion into products via a proton abstraction from the selenamide. Such a process is not possible for ebselen as the nitrogen atom does not have any available protons and thus it was apparent that the chemistry of the full molecules could not be captured using truncated models of their structure. Therefore, investigation of the full molecules was necessary.

Owing to the greater computational cost of modeling the full structures, in particular ebselen diselenide, geometry optimizations were performed with Becke's three-parameter exchange functional (B3) in conjunction with the correlation functional proposed by Lee, Yang and Parr (LYP) using the 6-31G(d,p) Pople basis
set. This level of theory is computationally less expensive than that used for the model structures but was shown to be reliable for the prediction of organo-selenium geometries and energetics [36]. Accurate energies were obtained for all structures via single-point calculations using the 6-311+G(3df,3pd) Pople basis set with the B3LYP method. Aqueous solvent effects were again incorporated implicitly with single-point calculations using the CPCM model at the B3LYP/6-311+G(3df,3pd) level. In addition, for the selenol anion reaction, diffuse functions were included on heavy atoms for the geometry optimizations and frequency calculations as well as in the transition state searches.

In the model compound study, the selenolate anion was shown to be more active towards the reduction of hydrogen peroxide than the neutral selenol. In addition, aromatic selenols have an experimental pK_a of approximately 6, again suggesting that at physiological pH (~7) the anion is the most likely species to be present; however, it was also prudent to probe the likelihood of the selenol zwitterion as a contributor to the GPx activity of ebselen selenol. Therefore, the conversion of the neutral selenol into the zwitterion was investigated (Figure 20.2).

Calculations on this system show that the zwitterion is unlikely to be the most active reducing agent as it lies on an unstable potential energy surface. The zwitterion is nearly equal in energy to the transition state between it and the selenol and thus is expected to be short-lived if produced at all. Sarma and Mudge have also experimentally determined that the ebselen selenol zwitterion is an unstable isomer of the neutral selenol [95]; therefore, the neutral selenol was not included in the full molecule investigation, only the selenolate anion.

The predicted solution-phase Gibbs energy barriers for the reduction of hydrogen peroxide by ebselen, the selenolate anion and ebselen diselenide are 36.8, 32.5 and 38.4 kcal mol^{-1}, respectively. Both the gaseous and solution-phase barriers are
indeed lower than for the case of the model compounds with the exception of the
diselenide, which exhibits similar barriers. Although the barriers are still over-
estimated with respect to the experimental values, there is a qualitative agreement
between the results and experiment in that the selenolate anion is predicted to be the
most active of the three.

Mechanistically, all three reactions proceed via a similar proton shift mechanism
(Figure 20.3). The incoming hydrogen peroxide molecule is oriented with one end
facing the substrate selenium atom. The proton on this end of the peroxide is
transferred to the neighboring oxygen atom while the selenoxide bond is formed,
resulting in a simultaneous proton transfer/selenoxide bond formation.

To probe in more detail the reasons for the higher activity of the selenolate anion,
the topology of the electron density was analyzed using the quantum theory of atoms
in molecules (QTAIM) [96]. QTAIM provides a tool with which one can unambigu-
ously partition any molecular system based on the topology of its electron density
into regions of space that define each atom in the molecule. A consequence of this
partitioning is that the properties of the atoms can be summed quantitatively to yield
the total value of that property for the full molecular system. Therefore, one can decompose any such molecular property into the individual atomic contributions. The implications of this have been observed empirically for many decades as the transferability of the properties attributed to atoms and functional groups between different molecules.

Decomposition of the molecular energy into its atomic components for the reactant complex (RC), transition state (TS) and product complex (PC) along a particular reaction coordinate affords the construction of an atomic reaction energy profile as shown in Figure 20.4. These profiles outline the atomic energy changes throughout the course of the reaction and in this particular case indicate a significant destabilization of the selenium atom and corresponding stabilization of the peroxide oxygen atoms in proceeding both from the reactant complex to the transition state and from the transition state to the product complex. The differences between the energy profiles of the three species are very subtle; however, the selenium atom in the case of the selenolate anion is clearly far less destabilized than in the case of the other two species despite the peroxide oxygen atoms having a very similar profile in all three species.

An explanation for this behavior is found in the decomposition of the electronic charge of each system. Analysis of the electronic population data for each case shows that the selenium atom loses 0.94–1.02 e of electronic charge by being oxidized, which is entirely transferred to the two peroxide oxygen atoms. In all cases, the oxygen atom of the resultant water molecule (O_w) recovers the majority of electronic charge (~60%) while the selenoxide oxygen (O_s) recovers the rest (~40%). The charge transfer for each case is slightly different though in terms of when it takes place. For the ebselen and ebselen diselenide systems, the selenium atom loses 0.36 and 0.30 e
of electronic charge at the TS, respectively, and loses an additional 0.58 and 0.72 \( e \) of electronic charge at the PC, respectively.

The selenium atom of the selenolate anion, however, has a greater electron population to begin with, which facilitates the loss of 0.47 \( e \) of electronic charge at both the TS and the PC. This reflects the higher propensity of an anion to be oxidized and elucidates the energy profile of the selenolate and the lower barrier to oxidation.

Sarma and Mugesh [95] have also used DFT to provide valuable supplemental data for their experimental investigation into the role of thiols in the catalytic cycle of ebselen and some ebselen analogues [97]. The structures of ebselen and several analogues were optimized with the B3LYP/6-31G(d) method with the effect of solvation in an aqueous medium incorporated using the isodensity polarizable continuum model (IPCM) [98]. Subsequent calculations were used to investigate the \( ^{77}\text{Se} \) chemical shifts using the GIAO method [99] and atomic interactions using the natural bond orbital (NBO) method [100].

Reduction of peroxides by the selenol form of ebselen is accompanied by a series of additional steps analogous to the catalytic cycle of GPx itself (Scheme 20.2). Sarma and Mugesh have shown that this process is further complicated by thiol exchange reactions whereby an incoming thiol that could potentially react with the selenol-sulfide (2) intermediate will generally prefer to attack the more nucleophilic selenium atom, yielding an exchange of the thiol substituents and no net reaction [95]. Thiol exchange has therefore been implicated in the relatively low GPx-like activity of ebselen, depending upon the thiol. These results have been supported by the theoretical work of Bachrach et al. [101]. In that study, various nucleophiles (HS\(^-\), CH\(_3\)SH, HSe\(^-\), and CH\(_3\)Se\(^-\)) and substrates (R\(_2\)SSeR\(_2\) and R\(_2\)SeSeR\(_2\) with R\(_1\) and R\(_2\) = H or Me) were used to model gas-phase substitution at selenium and sulfur in diselenides and selenol sulfoxides. Using MP2 and B3LYP, the PES of each reaction was investigated and in all cases it was found that nucleophilic attack at selenium is both kinetically and thermodynamically preferred over attack at sulfur. The implication of these results is that one needs to tackle thiol exchange reactions to design new, more effective GPx mimics.

20.3.2.2 Substituent Effects on the GPx-like Activity of Ebselen

Experimental investigation into potential organo-selenium therapeutic agents is often accomplished by generating a reasonably high number of unique organo-selenium compounds and testing them using various prescribed assays. The results will point to promising candidates for future drug molecules; however, a key disadvantage of such a methodology is that a proper understanding of the mechanism of action of any particular compound may be lost; that is, a full understanding of why a compound yields desired properties or not may not be fully explored and consequently the method may fail to uncover structures beyond the scope of the study whereas a knowledge-based approach could succeed in doing just that.

Consider the case of ebselen with a nitro substituent adjacent to the selenium atom. Experimentally this compound exhibits a GPx activity that is nine times greater than ebselen itself [102]. It is tempting to attribute this difference to the electron-withdrawing nature of the NO\(_2\) and, indeed, this has been done [27]; however, in the
oxidation of organo-selenium compounds by peroxide it has been shown that there is a significant reduction in electron density at the selenium atom [88] and thus an electron-withdrawing substituent would not be expected to aid such a process.

We have therefore investigated substituent effects on the GPE-like activity of the ebselen molecule with the goal of uncovering a more feasible explanation for the observed increase in activity of the nitro derivative [103]. A series of seven structures (Figure 20.5) were chosen to probe the electronic and steric effects of substituents on the ebselen framework and the transition states of the reactions of each with hydrogen peroxide were located. Reactant complexes, product complexes and transition states were optimized using the B3LYP/6-31G(d,p) and the topology of the electron density was analyzed using QTAIM. Table 20.2 summarizes the results.

Any structure having a substituent in the R₄ position (i.e., 17–20) exhibits a significantly larger barrier to oxidation. While the decomposition of the electronic charge of the system into its atomic components indicates that the electron-withdrawing and electron-donating substituents do indeed contribute to the charge on the selenium atom (q₉₀), the charge transfer during the reaction (indicated by Δq) is

<table>
<thead>
<tr>
<th>Table 20.2</th>
<th>Electronic energy barriers (ΔEₑ) electronic charges on selenium (q₉₀), changes in electronic charge on selenium and each peroxide oxygen atom (Δqₑ, Δqₑ, Δqₑ) and dihedral angles in the TS for the direct oxidation of ebselen and several derivatives by hydrogen peroxide.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td>ΔEₑ (kcal mol⁻¹)</td>
</tr>
<tr>
<td>1</td>
<td>25.7</td>
</tr>
<tr>
<td>15</td>
<td>22.8</td>
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<td>16</td>
<td>26.6</td>
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<td>17</td>
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<td>18</td>
<td>33.1</td>
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<td>19</td>
<td>30.9</td>
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<tr>
<td>20</td>
<td>30.9</td>
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virtually unchanged in all cases. There is, however, a stark contrast between the geometry of the transition state structures for cases where the substituent is in the R4 position (i.e., 17-20) and for cases where the substituent is anywhere else, as is illustrated by the dihedral angles in Table 20.2. These data strongly suggest a steric effect induced by substituents located close to the selenium atom that hinder the approach of incoming peroxides and thus raise the overall barrier of the reaction.

In the context of the full catalytic cycle of ebselen (Scheme 20.2) it is apparent that while a substituent near the selenium atom will hinder the approach of a peroxide molecule in the direct oxidation reaction (1 → 0), it must also hinder the approach of a thiol in the selenosulfide intermediate and thus prevent thiol exchange. Since it has been established that thiol exchange is to blame for poor activity in the case of ebselen, the experimentally observed increased activity of the nitro derivative of ebselen can be attributed to the steric effect of the substituent and thus a modification of the local steric environment of the selenium atom provides a tool for overcoming thiol exchange and increasing the GPX-like activity of the parent compound.

One might also expect that altering the framework of the parent compound would be advantageous to overcome thiol exchange. A much less obvious GPX mimic was discovered in 1,3-dihydro-1-methyl-2H-imidazole-2-selenol (MSeI, Figure 20.6) when Mugest et al. found, experimentally, its GPX activity to be much greater than that of ebselen [56, 104]. Soujanya et al. subsequently carried out a B3LYP/6-31G(d) investigation into its mechanism [105]. The full catalytic cycle analogous to that in Scheme 20.2 was studied with MSeI as the substrate and, despite calculating prohibitively high barriers for a catalytic process, it was shown that the Mulliken charges on the sulfur and selenium atoms of the seleno-sulfide intermediate were 0.434 and −0.390, respectively, suggesting that the imidazole framework may provide a basis to overcome thiol exchange in such systems. Any substrate framework that has the potential to enhance nucleophilic attack at sulfur versus selenium in the selenosulfide intermediate affords the opportunity to overcome thiol exchange and, therefore, has a higher potency as a GPX mimic. This may explain the observed high GPX-like activity of MSeI and, consequently, give new insight into effective GPX mimics.

20.3.2.3 Effect of the Molecular Environment on GPX-like Activity
An intuitive choice for a GPX mimic would be the Sec residue itself. Cardey and Enescu have studied the reduction of hydrogen peroxide by selenolate and thiolate [106] as well as the anions of Sec and Cys [107] theoretically. This was with the goal of uncovering details regarding what the effect of the immediately bonded environ-

![Figure 20.6](image)

Figure 20.6 Structure of 1,3-dihydro-1-methyl-2H-imidazole-2-selenol (MSeI).
ment of the selenium and sulfur atoms were that contributed to the overall activity of GPx. In their study, the integrated molecular orbital + molecular orbital (IMOMO) method [108] was employed, which has the ability to combine two calculations at two different levels of theory, analogous to the QM/MM methods employed for the full GPx protein (see above). In an IMOMO calculation, one chooses a high-level method to be applied to a restrained part of the quantum system and a lower level to be applied to the entire system; in the case of Carney and Enescu, QCISD(T) and MP2 were chosen, respectively. The complexes and transition states of each reaction with the Sec and Cys residues were optimized using the MP2/6-311+G(d,p) method in the gas phase, and in the aqueous phase with the use of a PCM dielectric, and the energetics of the reaction were subsequently calculated using the IMOMO method.

The results showed that Sec and Cys generally have very similar barriers to oxidation by hydrogen peroxide but are highly sensitive to the external dielectric modeling the effect of solvent. In addition, conformations of the amino acids that allowed for an intramolecular interaction between the peroxide and the NH group of the amino acid significantly reduce the reaction barrier. This indicates that the molecular environment is important for optimizing the efficiency of peroxide reduction with these species and reinforces the role played by the surrounding active site in the natural GPx enzyme as well as the direct interaction with solvent.

Because of the similarity in the overall GPx-like catalytic cycle of most small-molecule GPx mimics much information can be gained from studying a relatively small number of representative species. Many GPx mimics including ebselen are based on an aryl selenol framework and, as such, phenylselenol is a good approximation to most general aryl selenols. Bayse has used phenylselenol to study the effect of the theoretical treatment of the surrounding medium on the catalytic activity of aryl selenols [109]. Most quantum chemical calculations on such systems employ some adaptation of the polarizable continuum model where the effect of solvent is modeled by placing the system in a cavity with an external dielectric constant equal to that of the chosen medium, usually water. The advantage of such treatment is that it is relatively simple and robust; however, this comes at a cost of neglecting potentially important interactions with nearby solvent molecules. Clearly, these interactions are particularly important in the case of reactions where the solvent plays a direct role in facilitating some part of the process, say proton exchange. To capture such interactions, one must explicitly include the solvent in the calculation and so Bayse has constructed a network of water molecules to surround the substrate phenylselenol and each intermediate along its GPx-like catalytic cycle to allow proton exchange during the cycle to occur in a concerted process via the solvent.

These networks were constructed using two, three and four water molecules and each complex and transition state was optimized using the mPW1PW91 functional [42] in conjunction with relativistic effective core potentials. It was shown that when the solvent network facilitates proton exchange, the transition states for each step do not have to adopt the highly constrained geometries necessary for analogous gas-phase processes. As a result, the Gibbs energy barriers are significantly lowered and thus it is evident that explicit solvation is a key factor in obtaining realistic barriers for
the catalytic reduction of peroxides by small-molecule GPx mimics, just as it is for the full GPx enzyme.

20.4 Summary

In this chapter, we have presented a brief review of the quantum mechanical approaches to selenium biochemistry that have appeared over the past two decades. Much of the work focuses on understanding the mechanism of action of GPx and GPx-like small molecules to design potential therapeutic agents such as ebselen, which has been studied in great detail. It has been demonstrated that the role played by the molecular environment in the catalytic cycle is significant both for the entire GPx enzyme as well as for small molecule mimics of GPx.

Theoretical studies of GPx have helped to elucidate details of its structure and have answered questions concerning the presence of water molecules in the active site and what catalytic role these water molecules and neighboring residues of Sec play in the enzyme’s function. Specifically, nearby groups help to stabilize transition states and facilitate proton transfer in the reduction of hydrogen peroxide, which prevents the formation of highly strained transition structures. Such activity has also been found for water in theoretical studies of small molecule GPx mimics, indicating a strong contribution from solvent and the need for explicit solvation to achieve reasonable barriers for these processes.

Small molecule GPx mimics may also suffer from thiol exchange reactions whereby the catalytic activity is stalled by a shutting of thiol substituents. Several theoretical studies have shown potential avenues for overcoming thiol exchange reactions and, therefore, may lead to more effective GPx mimics and therapeutic agents. Such studies have shown that thiol exchange may be prevented through steric interference by substituents at specific sites of the ebselen structure and by electronic modification of the selenosulfide bond by using imidazoles as the catalytic substrate.

References
