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Review Article

Organochalcogen peroxidase mimetics as potential drugs: a long story of a promise still unfulfilled

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ABSTRACT

Organochalcogen compounds have attracted the interest of a multitude of studies to design potential therapeutic agents mimicking the peroxidase activity of selenium-based glutathione peroxidases (GPx's). Starting from the pioneering ebselen, various compounds have been synthesized over the years, which may be traced in three major classes of molecules: cyclic selenenyl amides, diaryl diselenides, and aromatic or aliphatic monoselenides. These compounds share common features and determinants needed to exert an efficient GPx-like activity, such as polarizing groups in close proximity to selenium and steric effects. Nonetheless, the reactivity of selenium, and tellurium as well, poses serious problems for the predictability of the biological effects of these compounds in vivo when used as potential drugs. These molecules, indeed, interfere with thiols of redox-regulated proteins and enzymes, leading to unexpected biological effects. The various chemical aspects of the reaction mechanism of peroxidase mimetics are surveyed here, focusing on experimental evidence and quantum mechanics calculations of organochalcogen representatives of the various classes.

Contents

Introduction

More than 30 years ago it was established that the enzymatic activity of glutathione peroxidase (GPx) is given by selenium [1–3], incorporated into the enzyme as the amino acid selenocysteine [4–6]. Biochemical [7], kinetic [8], and crystallographic [9] studies on the bovine cellular enzyme have suggested that the reduction of hydroperoxide by GPx is a cyclic process and whenever the selenocysteine was exchanged to cysteine in a GPx, the specific activity dropped by 2 to 3 orders of magnitude [10,11]. In the overall process of reduction of hydroperoxides catalyzed by GPx, 2 equivalents of glutathione are oxidized to the corresponding disulfide, whereas the hydroperoxide is reduced to its corresponding alcohol and water. The mechanism involves three steps [11–13]: (1) the selenol (E–SeH) is oxidized to selenenic acid (E–SeOH); (2) E–SeOH reacts with glutathione (GSH) to form a selenenylsulfide intermediate (E–SeSG); (3) a second GSH regenerates the initial E–SeH by attacking the E–SeSG and releasing oxidized glutathione (GSSG) (Scheme 1).

The GPx mechanism has been verified experimentally over the past decades [14–16], except for the elusive selenenic

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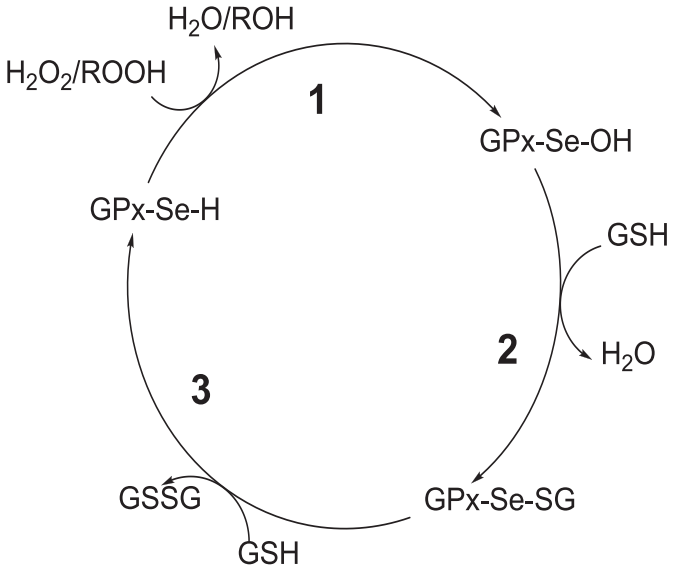


Scheme 1.

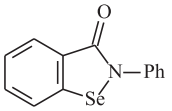
intermediate; in fact, selenenic acids have been isolated in only a few cases in special bowl-shaped molecular architectures [17]. More recent computational studies on GPx corroborated the mechanism sketched in Scheme 1 [18,19], although some aspects of the enzymatic activity are still under debate.

Various synthetic molecules have been designed with the aim to mimic the peroxidase activity of the GPx aiming to decrease the peroxide tone, thus having a pharmacological effect. The first and most studied molecule has been ebselen[1] [20,21], whose peroxidase activity has been measured on various hydroperoxide substrates [22] showing rate constants for both steps of the cycle by orders of magnitude lower than those calculated for glutathione peroxidase enzyme.

Having demonstrated that the catalytic mechanism is similar to that of GPx, its potential benefit as catalyst of hydroperoxide reduction inspired several studies on the discovery of novel molecules with enhanced reaction rates [23].

These compounds can be classified, as proposed by Bhabak and Mugesh [24], into three major categories: (i) cyclic selenenyl amides, (ii) diaryl diselenides, and (iii) aromatic or aliphatic monoselenides. Numerous studies have been published on these mimics and their antioxidant effects using both experimental and in silico approaches to unravel reaction mechanisms. Density functional theory (DFT) techniques, which are used to investigate the electronic structure of atoms and molecules at the quantum mechanical level of theory, have been extensively employed to describe the steps of the catalytic cycle. Computer-aided analysis also proved to be instrumental in defining specific paths for the enzyme-like activity of these compounds. Because most of these studies focused on a given set of molecules or on a single step of the catalytic cycle, the deduced information remains limited, because the catalytic activity does not rely on a single step, even if rate-determining, but is rather the outcome of the whole mechanistic path encompassing all the transition states and intermediates.

[1] WelterAndre,Christiaens Leon,Wirtz-Peitz. Patent No. EP0044453 (A2)—1982-01-27; New benzisoselenazolones, process for producing the same and pharmaceutical preparations containing the same. Cologne: A.Nattermann & Cie.



Scheme 2.

Group I: cyclic selenenyl amides

The most popular GPx mimic is certainly ebselen, i.e., 2-phenyl-1,2-benzisoselenazol-3(2*H*)-one [20,21] (Scheme 2).

Ebselen (**1**) falls into the category of the selenenyl amides and most of its biological activities (antioxidant, anti-inflammatory, and anti-cancer) have been attributed to its ability to mimic the enzymatic properties of GPx. The most likely path of the catalytic cycle of ebselen is colored in black in Scheme 3. The first step in ebselen-catalyzed reduction of hydroperoxides involves a ring opening by 1 equivalent of thiol to produce a selenenylsulfide intermediate (**2**). The selenol (**3**) is then generated by a second equivalent of thiol in a thiol–disulfide exchange reaction. The selenol reacts with the hydroperoxide to form a water molecule and the selenenic acid intermediate (**4**) that further reacts with a third equivalent of thiol to form water again and selenenylsulfide (**2**). Ebselen is ultimately regenerated by dehydration from **4.**

Antony and Bayse [25] have very recently modeled this mechanism employing a DFT solvent-assisted proton exchange approach and reported the following activation energies for the four reaction steps in water: $\Delta G^{\ddagger}_{1,2}=8.4$ kcal mol^{-1}, $\Delta G^{\ddagger}_{2,3}=31.7$ kcal mol^{-1}, $\Delta G^{\ddagger}_{3,4}=12.8$ kcal mol^{-1}, and $\Delta G^{\ddagger}_{4,2}=13.1$ kcal mol^{-1}; the activation energy calculated for the dehydration is $\Delta G^{\ddagger}_{4,1}=28.5$ kcal mol^{-1}; methanethiol and methyl hydroperoxide are employed in this study as thiol and hydroperoxide, respectively. The initial reduction (**1**→**2**) is the preferred path under most conditions: ebselen reacts easily with GSH and other thiols even at low temperature [26] and in vivo it is prevalently found bound to cysteine thiols of serum albumin [27]. The rate-determining step of the cycle is the formation of **3.** Sarma and Mugesh [28] studied this step of formation of the selenol in more detail and demonstrated that the thiol of the reducing substrate may attack either selenium or sulfur in **2.** In the first case, an exchange reaction occurs in which the former reducing substrate is released and the catalyst is not regenerated. In the second case, the reduction of the selenenylsulfide regenerates the selenol **3** together with the release of the disulfide by two molecules of the reducing substrates. These reactions have been rated as competitive and thus responsible for the poor GPx-like activity of ebselen. In particular it is the Se···O noncovalent interaction in **2** that favors nucleophilic attack of a thiol at selenium rather than at sulfur [28]. Bayse et al. [29] analyzed the strength of interaction between selenium and nitrogen- or oxygen-containing groups and concluded that also in GPx a carbonyl oxygen acts as a donor for the selenenic acid and the selenenylsulfide intermediates. Unluckily, the strength of the O···Se interaction in ebselen cannot be modulated by introducing ad hoc substituent groups in the amine [30,31]. Thus, Sarma and Mugesh [28] attempted to introduce a coordinating amine and other groups in the thiol cosubstrate with the aim of enhancing the catalytic activity of ebselen, by inhibiting the thiol exchange reaction. For example, using an oxazoline-based thiol (Scheme 4), they introduced an additional noncovalent Se···N interaction in the selenenylsulfide and were able to modulate the attack of the incoming thiol at the sulfur center.

Only very recently a study by Bayse and Pavlou [32] revealed that on aryl selenols weak Se···O or Se···N noncovalent interactions can be easily displaced, thus allowing the formation of selenol and the cleavage of the selenylsulfide as expected in a peroxidase catalytic cycle.