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Organoselenium Compounds as Glutathione Peroxidase Mimics

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Abbreviations:
$En\text{-}Se^-$, selenol form of the enzyme
En-SeOH, selenic acid form of the enzyme
En-Se-SG, selenenyl sulfide form of the enzyme
GPx, glutathione peroxidase
GSH, glutathione
GSSG, glutathione disulfide
GR, glutathione reductase
ROOH, alkyl hydroperoxide

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Abstract:

Glutathione peroxidase (GPx) is a selenoenzyme that protects biological membranes and other cellular components from oxidative damage by catalyzing the reduction of several types of hydroperoxides using glutathione (GSH) as the reducing substrate. It has been shown that the enzymatic activity of GPx is related to its selenocysteine residues that make up the catalytic site. Organoselenium compounds mimic the activity of (GPx) by introducing a selenium atom into an organic framework thereby recreating the catalytic or active center of the natural enzyme. They undergo the same catalytic reaction as GPx and are able to accept any cellular thiol. The organoselenium compounds are stabilized by the selenium–nitrogen (Se-N) interaction which is thought to play a major role in their antioxidant activity. Ebselen which was developed in the 1980s is often used as the model compound (in this class) for other structural analogs.

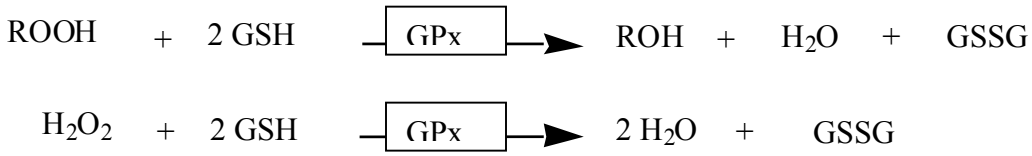
Introduction:

The glutathione peroxidase (GPx) pathway plays a major role in the antioxidant protection of mammalian cells during events of oxidative stress [1] as observed in some forms of human cancers. It functions to remove hydrogen peroxide (H_2O_2) and organic hydroperoxides (ROOH by coupling their reduction to water (H_2O) with the oxidation of glutathione (GSH). The cytosolic form of GPx can be inactivated by high levels of superoxide ($O_2^{\bullet -}$), hypochlorous acid and nitric oxide in the inflammatory response of endothelial cells [2] leading to episodes of hemolytic anemia, a clinical diagnosis of GPx deficiency.

Enzyme therapies for the treatment of various diseases have been limited due to the instability and immunogenicity of the endogenous forms. Several classes of compounds including the organoseleniums have been designed as GPx mimetics for studying the effectiveness of the pharmacological application of antioxidant therapy in the treatment of disease states related to oxidative stress; thereby, circumventing the intrinsic difficulties of using natural enzymes as drugs. Research has shown that some organoselenium compounds are effective antioxidant protectors in endothelial cells [2]. This review will focus on the properties and chemistry of GPx and organoselenium GPx mimetics.

Properties of Glutathione Peroxidase:

Glutathione peroxidase was first discovered in animal tissues in 1957 by Mills [3]. It is generally not present in high concentrations in plants or bacteria [1]. GPx is specific for GSH as a hydrogen (H^+) donor but acts on all types of hydroperoxides (H_2O_2 and ROOH) reducing them



Scheme 1

to H_2O (Scheme 1).

Glutathione peroxidases contain four identical protein subunits each of which contain one selenium (Se) atom at its active site. There are at least four types of Se containing glutathione peroxidases: (1) cytosolic GPx (GPx 1), (2) gastrointestinal (GI) GPx (GPx 2), (3) plasma GPx (GPx 3) and (4) phospholipid hydroperoxide GPx (Gpx 4) [4]. All show similarities in their structures. The bovine form of cGPx has been studied extensively and its active site is depicted in figure 1 where specific amino acid residues are highlighted (*i.e*. Arg (red), Gln (lilac), Phe (yellow) and the selenocystine center is in orange.

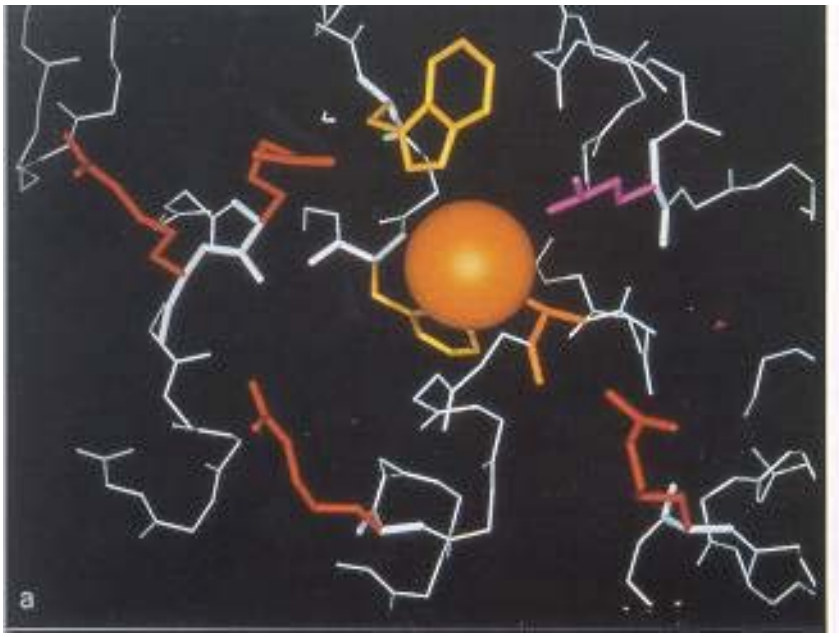


Figure 1. Active site structure of bovine GPx. (Adapted from [4])

During the catalysis of GPx, the selenol (En-Se^-) form of the enzyme reacts with a ROOH to give the selenic acid (En-SeOH) form of the enzyme (Reaction 1). GSH then binds to selenic acid to produce selenenyl sulfide (En-Se-SG) form of the enzyme (Reaction 2). A second GSH then binds to En-Se-SG to regenerate the selenol (Reaction 3).

$$\text{En-Se}^- + \text{ROOH} + \text{H}^+ \rightarrow \text{ROH} + \text{En-SeOH} \qquad (1)$$

$$\text{En-SeOH} + \text{GSH} \rightarrow H_2O + \text{En-Se-SG} \quad (2)$$

$$\text{En-Se-SG-GSH} \rightarrow \text{En-SeH-GSSG} \rightarrow \text{En-Se}^- + H^+ + \text{GSSG} \quad (3)$$

Selenium Properties:

Selenium as a free element is slightly electropholic and reacts with strong nucleophiles. The selenic acids also function as electrophiles. Selenium is similar in chemistry to sulfur of the group VI elements in the periodic table. It is actually present in the active site of GPx as selenocysteine, wherein the amino acid cysteine has a sulfur atom replaced by a Se atom (R-SeH instead of R-SH). A characteristic property of Se is its early changes of oxidation state. Se in comparison to other elements such as sulfur is more easily oxidized and reduced between valence states 2 and 4. Therefore, the lower redox potential of selenocysteine compared with cysteine is catalytically favorable for the En-SeH to En-SeOH form of the enzyme conversion.

Organoselenium Compounds:

Based on the properties of Se, several organoselenium compounds have been developed for thiol mediated destruction of peroxides. Certain features make Se compounds valuable when compared to sulfur compounds. The carbon selenium (C-Se) bond is weaker than the carbon sulfur (C-S) bond, and the relatively poor π overlap in C=Se bonds makes them more reactive than the C=S bond for instance, in cycloadditions [5].

A proposed antioxidant mechanism of the organeselenium compounds suggests that their peroxidase activity is related to the nitrogen base interaction with the selenium atom of En-SeOH to increase the nucleophilic attack of thiols [6].

Ebselen (Figure 2) was the first compound to be developed in this category that was

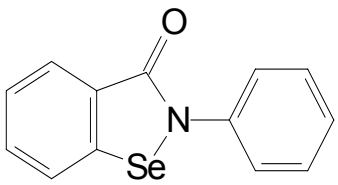


Figure 2. Structure of Ebselen. (Adapted from [7]).

successful at mimicking the structural active site of GPx [7]. An important property of ebselen is its inability to oxidize GSH in the presence of oxygen, which normally leads to the uncontrolled production of $O_2^{\bullet-}$ and other free radical species [8]. A hypothetical model of the redox cycle of ebselen (Figure 3A) is similar to that of endogenous GPx (Figure 3B). In figure 3A,

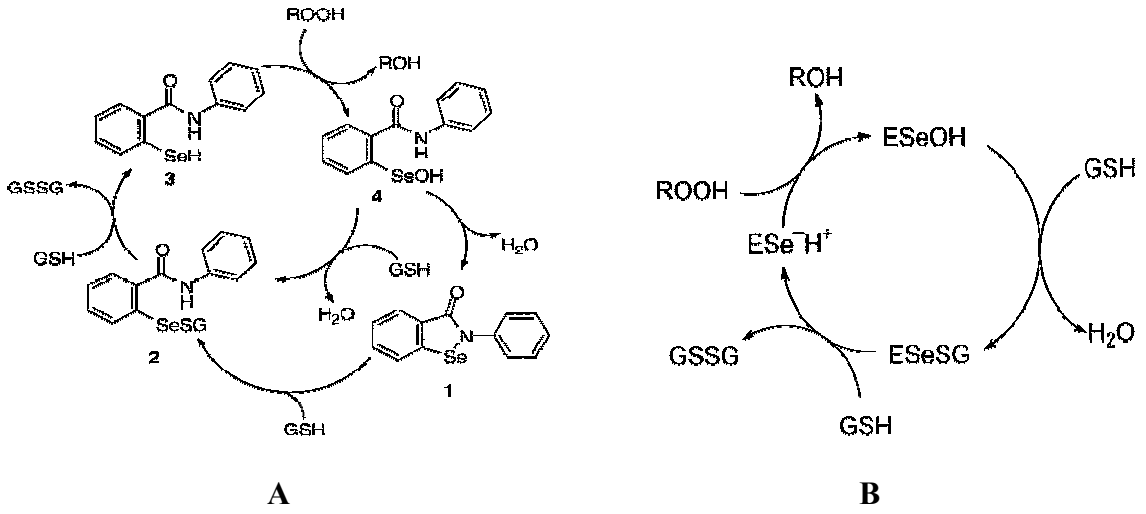
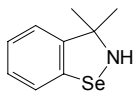


Figure 3. Redox shuttling of Se^{+2} and Se^{+4} using Ebselen (A) and GPx (B). (Adapted from [8])

ebselen (compound 1) reacts with GSH to produce the selenenyl sulfide (compound 2). Compound 2 reacts with a second GSH equivalent to produce a selenol (compound 3). Compound 3 reacts with the hydroperoxide to produce a selenic acid (compound 4). In figure 3B, the cycle is as previously described in reactions 1-3. The activation energy for the ebselen-catalyzed reaction is 55 kJ/mol per ºK, in comparison to 36.5 kJ/mol per ºK for the enzyme-catalyzed reaction.

Additional organoselenium compounds, BXT-51056 and BXT-51072, with GPx activity were reported in 1994 that were modifications of the basic ebselen structure (Figure 4) [8].

BXT-51056



BXT-51072

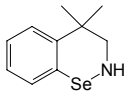
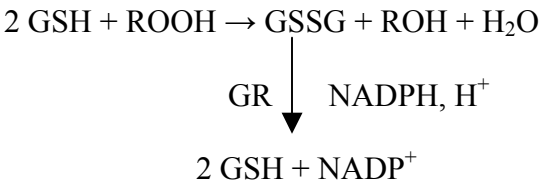


Figure 4. Chemical structures of new organoselenium compounds. (Adapted from [8])

The GPx activities for ebselen, BXT-51056, and BXT-51072 as determined by methods of Flohe and Gunzler [10] were 24, 19, and 47 respectively as expressed in nmoles nicotinamide adenine dinucleotide phosphate (NADPH) oxidized per minute [2]. BXT-51072 was tested in a Phase II A clinical trial involving ulcerative colitis patients where they showed significant improvement.

Assay for GPx:

The most common method used for measuring GPx is the continuous monitoring of glutathione disulfide (GSSG) formation. The principle is that GSSG formed during GPx reaction is continuously reduced by an excess of glutathione reductase (GR) activity that provides for a constant level of GSH (Scheme 2). The concomitant oxidation of NADPH is monitored spectrophotometrically at 340 nm [10]. The activity is calculated based on the Δ [NADPH] /min from the linear slopes of decreasing absorption using a molar extinction coefficient of 6.22 $M^{-1}cm^{-1}$.



Scheme 2

Conclusion:

Selenium compounds have made major advances since the early stages of development where toxicity limited studies into their pharmacological effectiveness. The introduction of organoselenium compounds reduced the toxicity since Se was no longer bio-available. It is now clear that many of the compounds in this class play an important role in biochemical processes ranging from antioxidants to anticancer agents and antiviral agents [9] based on the unique

properties of Se in the catalytic activities. Ebselen [9] and other organoselenium analogs such as BXT-51072 [2] show great therapeutic potential against various disease states.

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