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Glutathione Peroxidase mimics: Ebselen

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Abbreviations

DTNB, 5,5'-dithiobis(2-nitrobenzoic acid)
EnzSeH, selenol
EnzSeSG, selenenyl sulfide
EnzSeOH, selenenic acid
GPx, glutathione peroxidase
$GS^{\bullet}$, glutathiyl radical
GSH, glutathione
GSSG, glutathione disulfide
GR, glutathione disulfide reductase
HRP, horseradish peroxidase
$NADP^{+}$, nicotinamide adenine dinucleotide phosphate reduced form
NADPH, nicotinamide adenine dinucleotide phosphate
OFR, oxygen free radical
ROS, reactive oxygen species
ROOH, alkyl hydroperoxide
SOD, superoxide dismutases
TNB, 5-thiol-2-nitrobenzoic acid
Trx, thioredoxin
TrxR, thioredoxin reductase

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Abstract

The enzyme, glutathione peroxidase (GPx), is an antioxidant selenonenzyme. GPx can not only protect the cellular membrane but also other cellular components. There are five types of GPx: GPx-1 (cytosolic GPx), GPx-2 (gastrointestinal GPx), GPx-3 (plasma GPx), GPx-4 (phospholipids hydroperoxide GPx), and GPx-5 (epididymis-specific GPx) [2, 7]. Because of its specific redox properties selenium (Se) is an essential trace element. A selenol group in the active site of an enzyme instead of a thiol will lead to a dramatic catalytic advantage. A selenium compared to a sulfur can be more easily oxidized and reduced between Se(II) and Se(IV). Ebselen, one of the organic selenium compounds, protects against the cytotoxicity through its antioxidant capability. Although many GPx mimics have been developed, they often possess serious disadvantages, e.g., low activity, low solubility in water, and toxicity. In this review, I focus on ebselen, a GPx mimic, and its chemical properties.

Introduction

Ebselen, a seleno-organic compound with glutathione peroxidase-like activity is used in clinical trials against stroke. Moreover, it is a novel antioxidant that has been shown properties against oxidative stress. Ebselen, 2-phenyl-1,2-benzisoselenazol-3(2H)-one, is a synthetic selenium GPx mimic, not an enzyme. Ebselen was reported to reduce both phospholipids and cholesterol hydroperoxide to their alcohol product; therefore, it can suppress oxygen free radicals (OFR) formation. The higher efficiency of ebselen can prevent formation lipid peroxidation initiated radicals. Ebselen also has properties such as free radical and singlet oxygen quenching. Model experiments *in vitro* show that the protection against oxidative challenge afforded by ebselen can be explained largely by the activity as glutathione peroxidase mimic. The metabolism and disposition of ebselen is presented in this review. The main point is that the selenium is not bioavailable, explaining the extremely low toxicity observed in animal studies.

Properties of glutathione peroxidase

Glutathione peroxidase (GPx) is a mammalian selenoenzyme that catalyses the reduction of a wide variety of hydroperoxides that are known to generate highly reactive oxygen radicals, which cause damage to cell membranes. The principal role of selenium in mammals is as the catalytic site of the antioxidant enzyme GPx. The enzyme catalytic site

includes a selenocysteine residue in which the selenium undergoes a redox cycle involving the selenolate anion as the active form, which reduces hydroperoxides. This led to the development of organoselenium compounds designed to mimic this enzymatic activity *in vitro* [1, 3, 16].

GPx catalyses the reduction of peroxides using glutathione (GSH) as the reducing substrate according the following equations:

$$ROOH + 2GSH \xrightarrow{GPx} ROH + GSSG + H_2O \qquad \textbf{[equation 1]}$$

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2GSH + NADP^+ \qquad \textbf{[equation 2]}$$

Follow the change in absorbance of NADPH, $\epsilon_{340} = 6200\ M^{-1}cm^{-1}$

Properties of ebselen

Ebselen ($C_{13}H_9NOSe$), is a low molecular weight: (274.2) organoselen compound. The molecular actions of ebselen contribute to its anti-inflammatory and anti-oxidant properties, which have been demonstrated in a variety of *in vivo* model [1, 3].

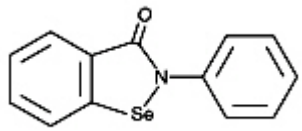
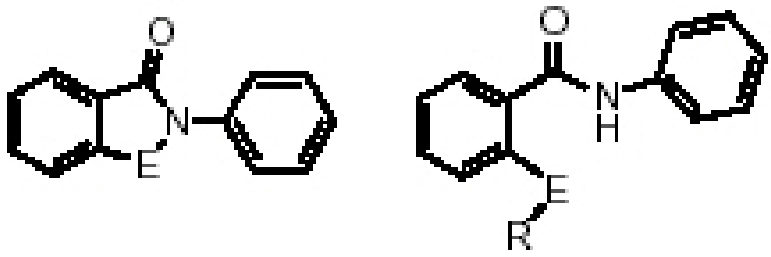


Figure 1: The structure of ebselen [7].

Easy changes in its oxidization state is a characteristic property of selenium (Figure 1). Studies on organoselenium compounds indicate that selenium in comparison to sulfur can be

more easily oxidized and reduced between valence state II and IV.

Ebselen is also an excellent scavenger with a higher rate constant than the sulfur analogue (Figure 2). To compare ebselen (**1**, a well known GPx mimic) is known to be an efficient catalyst than the sulfur analogue **2**. The redox ability of selenium is important when selenium undergoes oxidation-reduction reaction similar to the equation 1 and 2 (Figure 3). The efficiency of selenium compounds against oxidative stress correlate with their ability to deactivate of singlet oxygen. (Table 1). Ebselen inhibits both non-enzymatic and enzymatic membrane lipid peroxidation in cells. By directly reacting with protein thiol groups, ebselen also inhibits several enzymes involved in the inflammatory process, including lipoxygenases, nitric oxide synthases, NADPH oxidase, and protein kinase C.



1: E= Se
2: E= S

3: E= Se; R= Me
4: E= S; R= Me
5: E= Se; R= CH_2Ph
6: E= S; R= CH_2Ph

Figure 2: GPx mimics and their sulfur analogues [7].

Compound	k [$dm^3mol^{-1}s^{-1}$]
1	2.5×10^6
2	2.3×10^5
3	9.2×10^6
4	7.8×10^5
5	5.2×10^6
6	3.4×10^5

Table 1: The rate constants (k) of quenching singlet oxygen by Se-containing GPx mimics and their sulfur analogues [7].

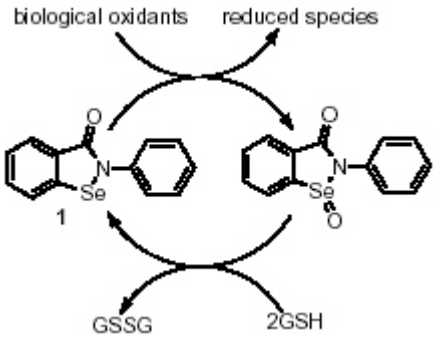


Figure 3: Redox shuttle between Se^{II} and Se^{IV} during the reaction of biological oxidants [7].

EPR spin trapping investigation of the effect of ebselen

The EPR signal, corresponding to the spin adduct of DMPO/thiyl ($GS^{\bullet}$) resulted from the reaction of DMPO with the $GS^{\bullet}$ radicals produced by HRP (horseradish peroxidase) enzymatic activity on GSH. The peroxidase-catalysed oxidation system can investigate the $GS^{\bullet}$ formation by adding H_2O_2 in the presence of HRP. This system was designed to assess the capability of ebselen to protect GSH from the oxidant attact by H_2O_2/HRP couple. Figure 4 shows the EPR spectrum characteriatic of the thiyl radical produced by enzymatic system

HRP/GSH/ H_2O_2 in the presence of 100mM DMPO [1, 9].

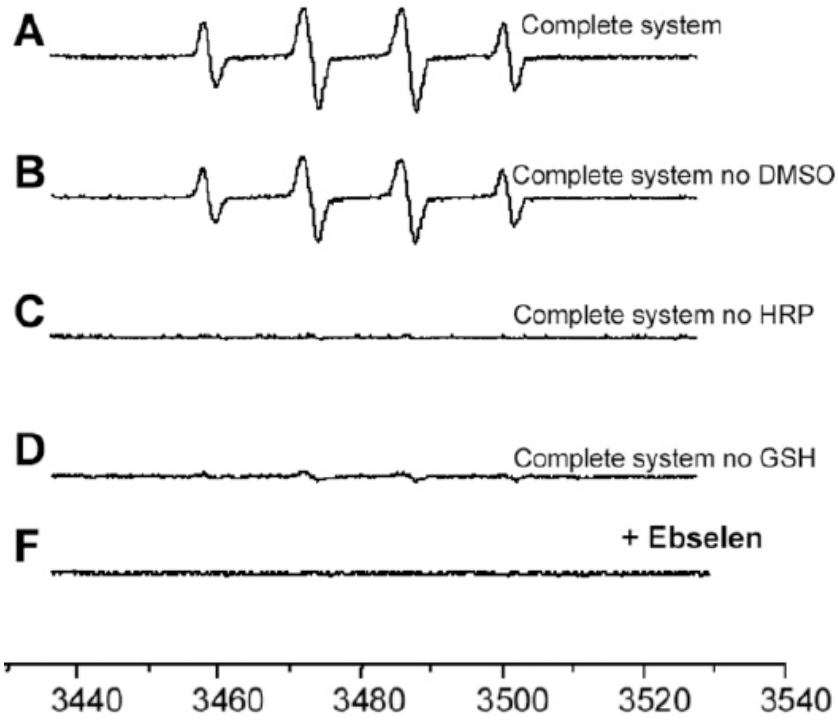


Figure 4: Thiyl ($GS^{\bullet}$) radical produced by enzymatic system and effects of ebselen. (A) Complete system HRP/ GSH/ H_2O_2 + DMPO. (B-D) Complete system without DMSO, HRP, or GSH respectively. (F) Same as (A) with ebselen [1].

The biological effects of ebselen

Because ebselen is effective against membrane hydroperoxides, it inhibits both nonenzymatic and enzymatic lipid peroxidation in cells and has anti-inflammatory activity in various animal models. Therefore, ebselen has been used in the treatment of patients with acute ischemic stroke. Ebselen was found to be an excellent substrate for mammalian TrxR (thioredoxin reductase) because the absorption at 340nm (A_{340}) decreased rapidly, and a highly efficient oxidant of reduced Trx, and it catalyzed H_2O_2 reduction (Figure 5) [6]. The Trx system (NADPH, Trx, and TrxR) is present in all living organisms. Because thioredoxin

reductase (TrxR) catalyzes the NADPH-dependent reduction of the active-site disulfide in oxidized Trx (Trx-S_2) to give a dithiol in reduced Trx. The mammalian enzymes also are NADPH-dependent lipid hydroperoxide reductases.

In figure 5, the reactions were complete after 3-4 min and showed no further decrease in A_{340}, demonstrating that the product was not redox cycling with oxygen. Visible precipitates in the cuvettes can be seen with higher concentrations of ebselen [6].

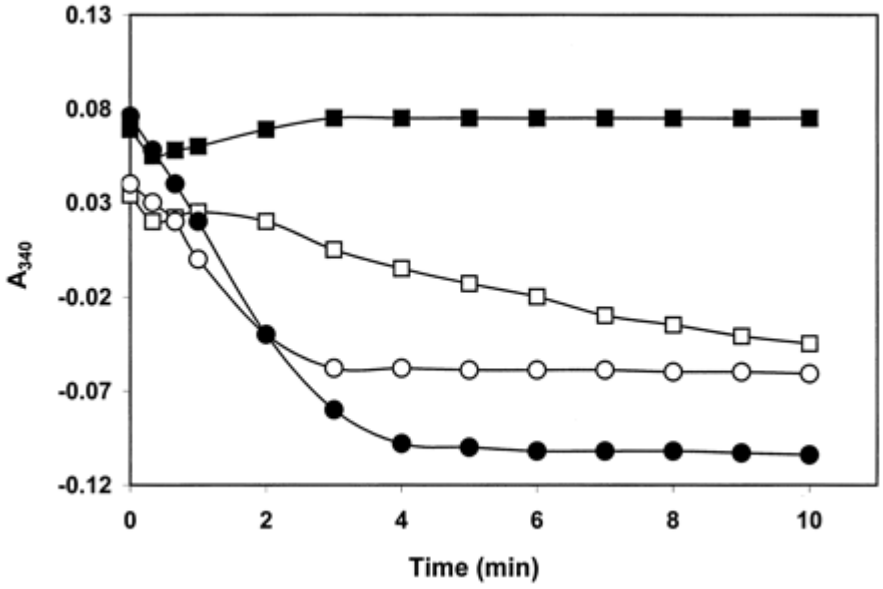
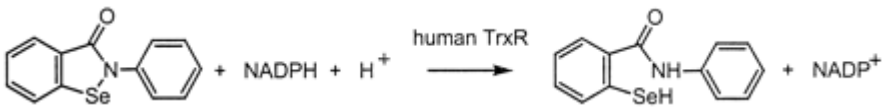


Figure 5: Reduction of ebselen by NADPH is catalyzed by human TrxR. Concentrations of 10µM (○, □) and 20 µM (●, ■) ebselen, and 100 uM NADPH was mixed with 10 nM (■, □), and 50 nM (●, ○) human TrxR.

Reduction of ebselen by NADPH catalyzed by the enzyme produces the selenol (Reaction 3) presumably via a short-lived isoselenazolone ring opened bound intermediate [6].



[Reaction 3]

[Reaction 4]

Excess of ebselen was found to react with the selenol forming an ebselen diselenide, which absorbs strongly at 340 nm and has a low solubility, giving rise to the precipitate and increase in A_{340}. Ebselen has no reaction with DTNB [6].

Summary

Ebselen has been shown involved in lots of effects such as anti-oxidation, anti-inflammatory, and free radical scavenger. These abilities can be attributed to its seleno-containing structure with GPx like activity. The peroxidase activity of ebselen operating via TrxR should eliminate H_2O_2 and lipid hydroperoxides, thus giving a mechanistic explanation for the effects in protecting cells from ischemic tissue damage. The clinical trials using ebselen have shown promising effects in acute ischemic stroke or delayed neurological deficits after aneurismal subarachnoid hemorrhage. TrxR and Trx are present in all the cells in our body and may be particular important in cells in nervous system for protecting against oxidative stress. However, high concentration of ebselen should be toxic by inhibition of the activit of Trx system.

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