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Ebselen as a Glutathione Peroxidase Mimic: Alias Chemical Chameleon Antioxidant

by

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Abbreviations:
GSH (glutathione)
GSSG (glutathione disulfide)
GPx (glutathione peroxidase)
PhGPx (phospholipid hydroperoxide glutathione peroxidase)

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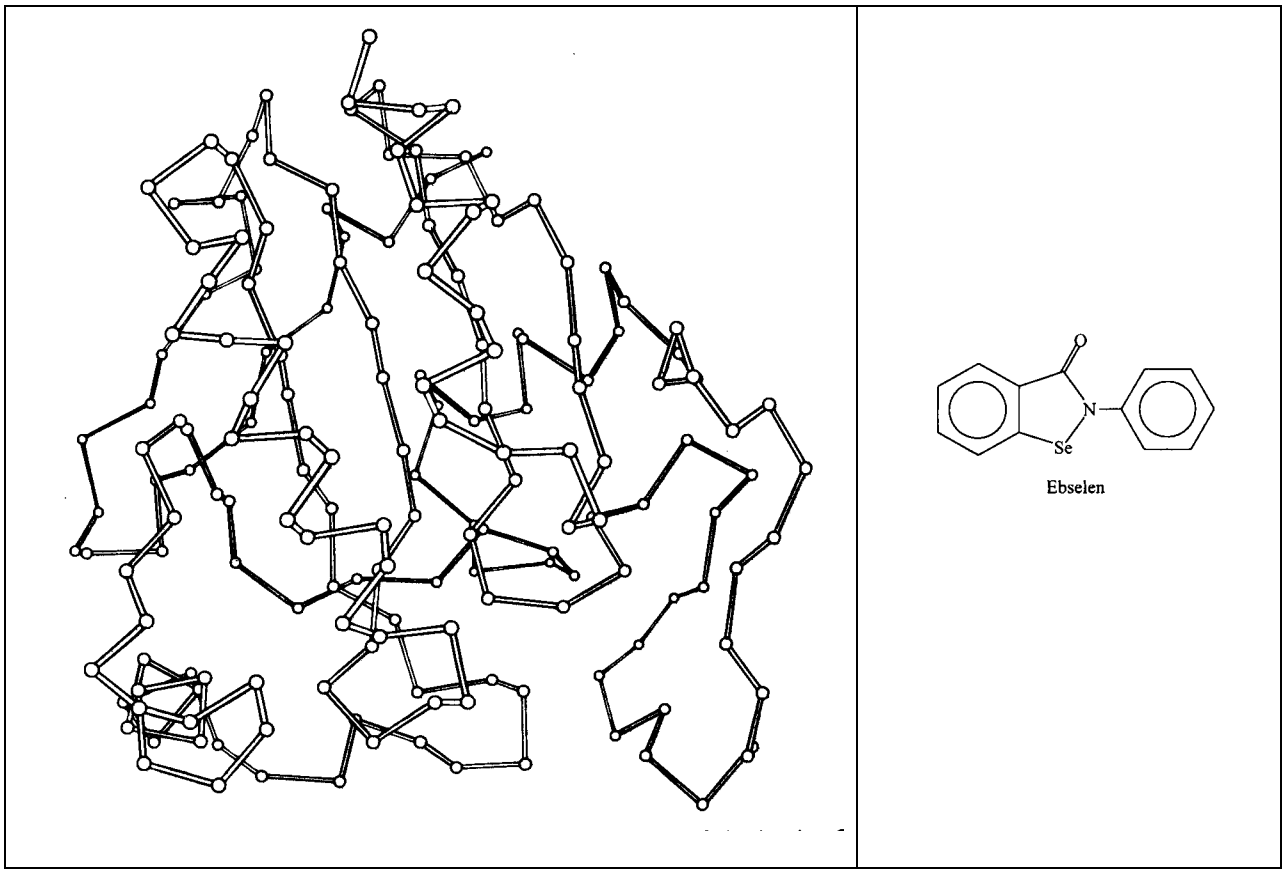
Abstract

Ebselen (2-phenyl-1, 2-benzisoselenazol-3 (2H)-one) is a glutathione peroxidase mimic that catalyzes the reduction of a hydroperoxide with the oxidation of a thiol. *In vitro* models indicate that the oxidative protection ebselen affords to cells is due to its glutathione peroxidase (GPx) mimic activity. This paper will describe ebselen's reactions, detection, and pharmacological potential.

Introduction

Glutathione peroxidase was discovered over 40 years ago in erythrocytes [11]. Around the same time, nutrition studies found selenium to be an important micronutrient. Selenocysteine is now known to be a required component of the active site of GPx [7]. Four versions of glutathione peroxidase have been found (classical erythrocyte GPx-I [11], PhGPx [24, 23],

plasma GPx [22,21], and GI-GPx) along with many selenopeptides and selenoproteins with unknown functions [Behne]. GPx-I is a tetramer with four active sites [6,26]. Ebselen (PZ 51) was first described in 1984 as a selenoorganic molecule with glutathione peroxidase mimic activity [13,25]:



(A) (B)

Figure 1. Glutathione peroxidase (A- the peptide backbone of a bovine GSH peroxidase subunit) [5] and ebselen (B- the synthesized drug**)** [7] structures are shown to make their size and complexity differences apparent.

The structural differences in these compounds contribute to ebselen's low specificity in comparison to GPx. Fewer conformational folds and an absence of amino acid residue charges frees ebselen from the multiple binding requirements of GSH peroxidase. The molocules' active sites, however, have been shown to have closely related mechanistic functions. An

understanding of ebselen chemistry and biochemistry clearly depends upon an understanding of glutathione peroxidase reactivity which will be elucidated in the following section.

GPx Reactivity

Three steps are considered to be involved in the enzymatic catalysis of GPx and PhGPx [5]. The active site for both of these molecules is a selenocysteine group [19]. The enzyme-bound selenocysteine reacts with an alkyl hydroperoxide to produce selenic acid and an alkyl hydroxide in reaction (1) [19]. In reaction (2), the selenic acid reacts with glutathione to produce selenosulfide (refered to as selenodisulfide in the reference) and water [19]. Reaction (3) transforms selenodisulfide back into the origional enzyme-bound selenocysteine through its reaction with another glutathione which also results in the formation of glutathione disulfide, GSSG [19]. In the stoichiometric reaction (4), the sum of reactions (1), (2), and (3) is illustrated [19].

$$\text{E-Cys-SeH} + \text{ROOH} \rightarrow \text{E-Cys-SeOH} + \text{ROH} \quad (1)$$

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

$$\text{E-Cys-Se-SG} + \text{GSH} \rightarrow \text{E-Cys-SeH} + \text{GSSG} \quad (3)$$

$$\text{Net:} \quad \text{ROOH} + 2\text{GSH} \rightarrow \text{ROH} + H_2O + \text{GSSG} \quad (4)$$

GPx Mimic Reactivity

A kinetic study [9] indicates that the above reaction can be catalyzed by ebselen through an analogous reaction scheme [19,5,27,25]. In this scheme (Figure 2), the ebselen selenol moiety is considered to be primarily responsible for the GPx activity of the GPx mimic [12]. Ebselen can use thiols other than GSH as substrates in this reaction (*i.e.* dithioerythretol [13], N-acetylcysteine [2], and dihyrolipoate [19]). Ebselen's relatively low enzymatic binding specificity in comparison to GPx is demonstrated through these examples [19]. The difference is at least partially due to ebselen's less complicated structure and smaller size.

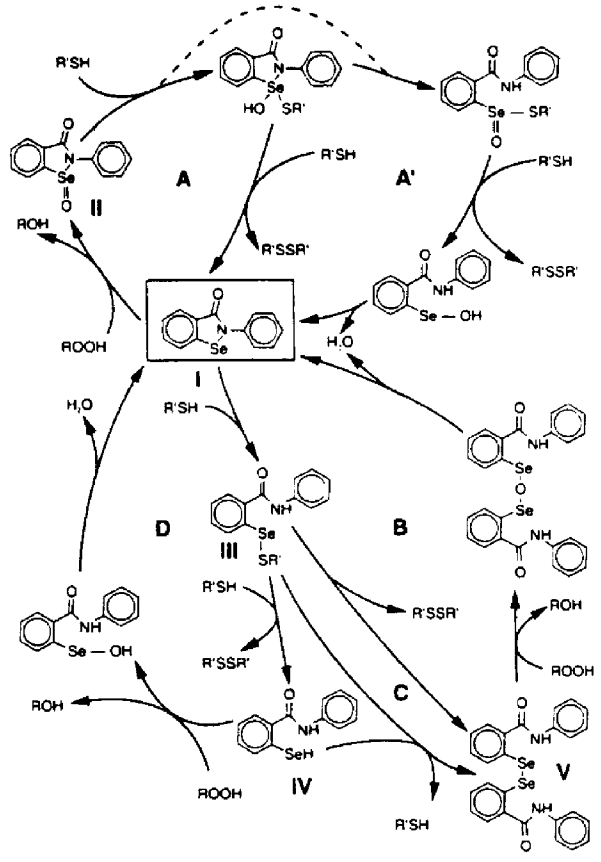


Figure 2. Ebselen shows a directly analogous reaction scheme to glutathione peroxidase. Thiols and hydroperoxides react with ebselen and it's metabolites in recycling loops (A-D). Primary compounds of interest are ebselen (I), ebselen selenoxide (II), ebselen selenenylsulphide (III), ebselen selenol (IV), and ebselen diselenide (V). Symbols include R (residues of polyunsaturated phospholipids, cholesteryl esters, cholesterol or any other organic hydroperoxide or hydrogen) and R' (glutathione, dihydrolipoate, *N*-acetylcysteine or thiol protein residue). Excess hydroperoxide over thiol favors cycle A. Cycles B, C, and D are preferred in other circumstances. Aprotic solvent environments favor cycle B, where aqueous systems favor a proportion of C and D dependant on the types of thiols present [17].

Interception of peroxynitrite

Addition	Rate constant[a] (M^{-1} s^{-1})	In vivo conc. (M)	Disappearance of $ONOO^-$ (s^{-1})	Remarks
Spontaneous decay	–	–	0.4	
Low-molecular-weight compounds				
Carbon dioxide	3.0×10^4 [26]	1×10^{-3}	30	Enhances tyrosine nitration and thiyl radical formation [27,28]
Glutathione	5.8×10^2 [39]	1×10^{-2}[b]	5.8	Formation of thiyl radicals [9]
Ascorbate	5.0×10^1 [45]	1×10^{-2}[c]	0.5	–
Ebselen	2.0×10^6 [30]	–	–	–
Metalloporphyrins	2.0×10^6 [38,39]	–	–	–
Proteins				
Myeloperoxidase	4.8×10^6 [46]	5×10^{-4}[d]	2400	Enhances tyrosine nitration [48]
Hemoglobin	2.5×10^4 [70]	5×10^{-3}[e]	125	–
Glutathione peroxidase[f]	8.0×10^6 [52]	2×10^{-6}[g]	16	–
Albumin	5.6×10^2 [60]	6×10^{-4}[h]	3.4	Formation of thiyl radicals [28]

Table 1. Selenol's role as a primary factor in the glutathione peroxidase activities of ebselen can be further demonstrated by looking at it's relatively high reactivity toward hydroperoxides with these second-order rate constants [17].

GPx Mimic Activity Detection

Coupled enzymatic assays, GSH removal assays, and hydroperoxide removal assays have all been used in the detection of GPx mimic activity [19]. The coupled enzymatic assay utilizes a rate-limiting test reaction with GSSG. NADPH loss is followed *via* absorbance spectrophotometry [13,9]. The GSH removal assay stops the reaction, then tests for remaining GSH *via* thionitrobenzoate formation with the addition of Ellman's reagent [25] or the formation of a monobromobimane adduct [2]. A typical version of the hydroperoxide removal assay utilizes the iron-thiocyanate compex [2,3] for GPx mimic activity detection.

Direct radical scavenging activity

The discovery of ebselen's radical scavenging abilities was made with pulse radiolysis studies following selenoorganic reactions with 1,2-dichloroethane radicals and halogenated peroxyl radicals [18]. The reaction of trichloromethyl peroxyl radicals and ebselen produced a rate constant (Table 2) within the same range as alpha-tocopherol (vitamin E) in similar circumstances [19, 18]. In spite of this, biological model systems using competition kinetics showed that ebselen radical scavenging was not effective [19, 10, 16]. One analysis was based on the inhibition of crocin carotinoid bleaching by hydroperoxyl radicals (Table 3) [10]

Antioxidant	k (10^8 M^{-1} s^{-1})
β-Carotene	15
α-Tocopherol	5.0
Ebselen	2.9
Ascorbate	2.0[a], 1.1[b]
Trolox	1.6
Urate	1.2
Methionine	0.2

Table 2. An antioxidant rate constant comparison upon reaction with trichloromethyl peroxyl radicals shows the relatively high activity of ebselen [18].

Compound	K_a
DPPD	7.
Trolox	1.
Probucol	0.
Chloropromazine	0
BHT	0
Ebselen	−0

Table 3. Radical scavenging activity is indicated by K_a/K_c (the ability of molecules to interact with hydroperoxyl radicals). K_a indicates the rate constant for antioxidant/free radical interactions. K_c indicates the rate constant for crocin/free radical interactions. Higher values indicate more interactions and, therefore, more radical scavenging activity [10].

Pharmacological potential resulting from the GPx mimic's prescence *in vivo*

Glutathione peroxidase mimics provide antioxidant protection against lipid peroxidation and are metabolized without evidence of significant toxicity [19]. The reduction of lipid hydroperoxides present in liposomes or lipoproteins is proposed as the mechanism leading to this protection [9,10]. As a more specific example, ascorbate and NADPH have been used *in vitro* as reductants with ebselen to protect against iron-induced lipid peroxidation [13, 8, 15]. Ebselen's role as an antioxidant is primarily based on its activity as a GPx mimic, and especially its role as a PhGPx, phospholipid hydroperoxide glutathione peroxidase, mimic [19, 10, 16]. The hydrophobicity of ebselen contributes to its effectiveness in antioxidant activity in and around membranes while its small size gives it the ability to diffuse and protect cellular compartments that are inaccessible to GPx [19]. Molecules from the ebselen catalytic cycles are eventually broken down and their products, including 4'-Glucuronyloxy-2-methylselenobenzanilide and 2-(Methylseleno)-benzoic acid are excreted in the urine, without any evidence of toxic side effects [19, 4]. In low concentrations, ebselen has been shown to inhibit enzymes involved in

inflammation (lipoxygenases, ·NO synthetases, NADPH, oxidase, protein kinase C, and H^+/ K^+-ATPase) [17].

Human cell experiments involving alimentary deficiency symptoms, which were found to be selenium-responsive, give further indirect evidence of ebselen's antioxidant benefits as they relate to it's selenium dependance [5]. A few symptoms which showed a positive response were: impaired hexose monophosphate shunts (erythrocytes), thrombasthenia (thrombocytes), slightly impared killing (neutrophiles), weakness/myalgy (skeletal muscles), and Keshan disease (heart muscles) [5].

Summary

The role of antioxidants in maintaining a primarily reducing environment within biological systems is supported through well characterized chemical reactivity *in vitro*. The mechanisms of ebselen metabolism pathways and transport *in vivo* are not well understood and there is still much work to be done in other areas, such as long term toxicity due to ebselen exposure, before clinical trials can be considered. Glutathione peroxidase mimic's flexability in substrate binding, combined with it's accessability throughout the cellular milieu, and a lack of significant toxicity could make it a potentially powerful and versatile therapeutic antioxidant.

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