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Phospholipid Hydroperoxide Glutathione Peroxidase (PhGPx) — An often overlooked antioxidant enzyme

by

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Abbreviations:

cGPx, cytosolic glutathione peroxidase;
giGPx, gastrointestinal glutathione peroxidase;
GPx, glutathione peroxidase;
GR, glutathione reductase;
GSH, glutathione;
GSSG, glutathione disulfide;
pGPx, plasma glutathione peroxidase;
PhGPx, phospholipid hydroperoxide glutathione peroxidase;
PLA_2, Phospholipase A_2.

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Abstract

Phospholipid hydroperoxide glutathione peroxidase (PhGPx), a selenium-requiring peroxidase, is a newly discovered member of the glutathione peroxidase family. It can directly reduce hydroperoxides of phospholipid, fatty acid and cholesterol in cellular membrane. It plays an important role in inhibition of lipid peroxidation. The structure of PhGPx, its kinetic mechanism and tissue distribution as well as its antioxidant function will be briefly reviewed in this paper.

Introduction

Phospholipid hydroperoxide glutathione peroxidase (PhGPx) was first described in 1982 [1] and later verified as a selenoprotein by sequencing [2]. It is a monomeric enzyme that contains one selenium atom at the active site as selenoccysteine. PhGPx belongs to the GPx family that includes other members: classical cytosolic GPx (cGPx), plasma GPx (pGPx) and GPx prevalent in gastrointestinal tract (giGPx) [3]. But in contrast to other members of GPx family, PhGPx can directly reduce phospholipid- and cholesterol- hydroperoxides in cellular membranes to corresponding alcohols at the expense of glutathione (GSH) [1, 4]. Thus, PhGPx plays a unique role in protecting cells against the damaging effects of lipid peroxidation. It is a crucial enzyme in the protection of cellular membranes against oxidative damage. This paper will discuss the structure of PhGPx, the kinetic mechanism and its tissue distribution as well as its antioxidant function.

Structures of PhGPx

PhGPx is a monomeric enzyme. The molecular weights of PhGPx in animal and human cells are 22-23 kDa and 18 kDa, respectively (measured by SDS-PAGE) [5, 6]. This enzyme contains one selenium atom, which is located in the flat depression of the enzyme surface. The selenium atom is involved in the catalysis and the active form is selenocysteine, which is similar to that of GPx. The cDNA encoding human testis PhGPx that is highly homologous with rat cDNA of PhGPx, has been cloned and sequenced (Figure I) [7].

There are two locations of PhGPx in cells: in the mitochondria, produced from long form PhGPx mRNA, and in the cytosol from a short from PhGPx mRNA [8]. That happens because the PhGPx gene has two transcriptional start sites [9]. The synthesis of a specfic form from of its mRNA is tissue dependent. In testis, a predominant long form mRNA directs the synthesis of a

197-amino acid protein (L-form) containing a potential mitochondrial targeting signal, 27-amino acids, at the N-terminius. This 27-amino acid sequence is highly conserved and directs the protein into mitochondria [9]. In the mitochondria, PhGPx plays a major role in protecting cells from oxidative injury [10] [11]. Somatic tissues primarily express the short-form PhGPx that is short of an N-terminal leading sequence and has 170-amino acid protein [9]. The short-form stays in the cytosol and can also protect cells from oxidative injury [9].

In contrast to GPx, the coding area of the PhGPx gene is composed of seven exons. Only the amino acid sequences encoded by the third and fifth exon are highly homologous to GPx sequences [12]. This suggests an identical catalytic mechanism of PhGPx and GPx.

Figure I. The nt sequence of the full-length human gpx4 cDNA and the deduced aa sequence. The nt probe (underlined sequence) used to screen a ?gt 11 human testis cDNA library. Clontech was prepared by PCR amplification of the whole library using primers based on the pPhGPx nt sequence. The stop codon is at 671-673 (ter). The TYR residue that is phosphorylated in pPhGPx is encoded by nt 446-448 (marked as ***). The boxed sequence in the 3'-*UTR* is the putative poly (A)-addition signal [7].

Enzymatic Function of PhGPx

1. Protection of PhGPx against lipid peroxidation:

The repair of intracellular LOOHs can be carried out by cytosolic glutathione peroxidase (cGPx) [17], phospholipid hydroperoxide glutathione peroxidase (PhGPx) [4], non-selenium GPx [8] or glutathione-S-transferase (GST) [19]. However, cGPx require the cooperative action of phospholipase A_2 (PLA_2) for the removal of LOOHs. PhGPx is able to directly reduce both phospholipid- and cholesterol-hydroperoxides in cell membranes [4,5]. The rate of removal of LOOHs by PhGPx has been estimated to be about four-orders of magnitude higher than the rate of removal by cGPx and PLA_2 [20].

It has also been found that PhGPx can detoxify membrane cholesterol hydroperoxide [4]. It has been found that cholesterol hydroperoxide decay is in first-order fashion ($k \sim 2.8\ h^{-1}$) over a 30-min period of incubation with GSH/PhGPx. By contrast, LOOH decay is biphasic, with an initial rapid drop over the first 5 min ($k \sim 19\ h^{-1}$), followed by a terminal slow reaction ($k \sim 3.6\ h^{-1}$). In the absence of PhGPx, peroxide loss is relatively slow ($k \sim 0.2\ h^{-1}$).

2. Reduction of thymine hydroperoxide by PhGPx

Thymine residues in DNA have the highest electron affinity and easily react with free radicals to yield thymine hydroperoxides [21]. It has been reported that human PhGPx has about 4 orders of magnitude higher activity on detoxification of thymine hydroperoxide than that of other selenium-dependent GPx and GST [21]. The reaction is shown below:

3. PhGPx may participate in cellular signaling pathway and may be involved in some inflammatory diseases [22, 23]. Because these results are unsure, details will not be discussed here.

Kinetics of PhGPx

The principal reaction catalyzed by PhGPx is: 2GSH + ROOH ? GSSG + H_2O + ROOH. The kinetic mechanism of PhGPx appears to be identical to that of glutathione peroxidase (cGPx). Both enzymes undergo a ping-pong reaction mechanism [5]. All selenium-dependant peroxidases catalyze the redox reaction where a hydroperoxide is reduced and two glutathione molecules are oxidized to the disuflide.

$$LOOH + PhGPx\text{-}Se^- + H^+ \longrightarrow LOH + PhGPx\text{-}SeOH \quad (1)$$

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

$$PhGPx\text{-}Se\text{-}SG + GSH \longrightarrow PhGPx\text{-}Se^- + GSSG + H^+ \quad (3)$$

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2GSH + NADP^+ \quad (4)$$

The mechanism for these reactions is shown in Figure II [6]. The first step is an oxidation of the selenol group of the enzyme by a hydroperoxide to form a selenenic acid derivative. The second step leads to the formation of covalent bonding between the sulfur of the GSH and the selenium of the enzyme. The last step is the regeneration of the reduced enzyme *via* second GSH that breaks the selenadisulfide bridge in E-Se-SG.

The kinetic of the reaction catalyzed by PhGPx also fits the Dalziel equation: $[E_0] / V = Ø_1 /[ROOH] + Ø_2 /[GSH]$. $[E_0]$ is the total enzyme concentration and V is velocity, $Ø_1$ and $Ø_2$ are the kinetic coefficients [12]. Since [GSH] is a constant and $[E_0]$ and [ROOH] are known, it is possible to measure $Ø_1$ and $Ø_2$: $Ø_1=1/k_{+1}$, $Ø_2=1/k_{+2} + 1/k_{+3}$, where k_{+1} is the rate constant for the oxidation of the enzyme in the presence of a hydroperoxide and k_{+2} and k_{+3} are the rate constants for the two reductive steps in the presence of GSH. Usually the rate constants k_{+2} and k_{+3} can not be differentiated. It is worthwhile to mention here, k_{+1} value for H_2O_2 is lower in reaction with PhGPx than with cGPx [5]. However, PhGPx can directly and rapidly reduce hydroperoxides of phospholipid, fatty acid, and cholesterol, which are not substrates for cGPx. Beside GSH, the donor substrate for PhGPx can be dithols and dithiothreitol, which are poor substrates for cGPx [13].

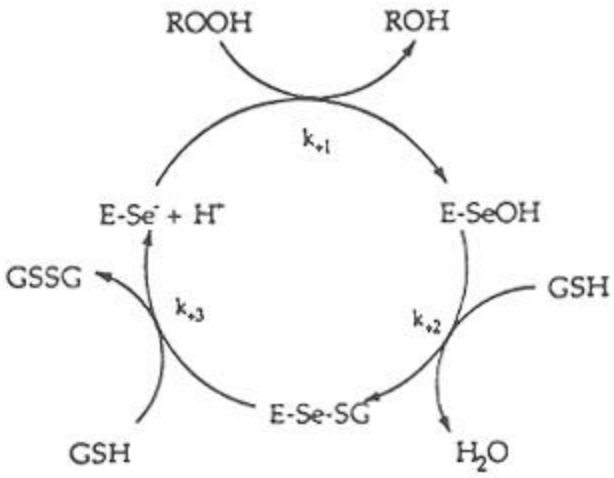

Figure II. The ping-pong mechanism for GPx and PhGPx [6]

Tissue Distribution of PhGPx

PhGPx has been found in all mammalian tissues [12] and the sequence of PhGPx is highly conserved between human, rat and pig [8]. However, the synthesis of a specific form is

tissue dependent. Rat testis contains 3.3 times more PhGPx activity in mitochondria than in cytosol, but in somatic cells, non-mitochondria PhGPx is much higher than the mitochondrial form [14]. The significance of this uneven distribution of PhGPx in testis is still unknown. It may be related to the spermatogenesis function [15]. It has been found that the long form, which can import into mitochondria is considered more potent than the short form in protection from oxidative injury because mitochondrial are a major physiological source of ROS. The long–form of PhGPx has been considered to play a major role in preventing oxidative injury to cells [16].

Summary

PhGPx, a member of glutathione peroxidase superfamily, is a selenoenzyme. It can directly reduce phospholipid and cholesterol hydroperoxides produced in the membranes in expense of two glutathione molecules. Two types of PhGPx have been identified, the cytoplasmic form (short form) and the mitochondrial form (long form). The mitochondrial form of PhGPx appears to provide more effective protection against oxidative damage than cytoplasmic one. PhGPx is an important enzyme in protection of cellular membrane lipid and DNA from peroxidation.

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