This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2003)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2003 Term

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Classic Glutathione Peroxidase

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For 77:222, Spring 2003
March 13, 2003

Paper III

Abbreviations:
GPx: glutathione peroxidase
$NADP^+$: nicotinamide adenine dinuclotide phosphate
NADPH: nicotinamide adenine dinuclotide phosphate reduced form
ROOH: Hydroperoxide
GSH: glutathione
GSSG: glutathione disulfide
SOD: superoxide dismutase
ROS: reactive oxygen species

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Abstract

Glutathione peroxidase (GPx) is a selenoenzyme that is a tetrameric protein of molecular weight ranging from 75 kDa to 100 kDa. GPx can catalyze the reduction of various hydroperoxides and hydrogen peroxide to lipid alcohol and water by glutathione. The GPx family contains four isozymes, GPx1-4. In this paper, the structure, chemistry, biochemistry and kinetic mechanism of GPx1 will be discussed.

Introduction

Reactive oxygen species (ROS) generated by oxidative stress are considered involving in many human diseases, such as aging, diabetes, stroke and many types of cancers. When ROS damage cell compounds such as lipid, protein, carbohydrate and DNA, they will induce the generation of hydroperoxides that have been demonstrated as harmful species to biological structure. Hence, the prevention of peroxidation is an essential process. There are several antioxidant defenses exist, including manganese superoxide dismutase (SOD), catalase, GPx, ascorbic acid, α-tocopherol, glutathione and β-carotene [1]. GPx play a key role in metabolizing H_2O_2, hydroperoxides by reducing reduced glutathione (GSH). GPx are enzymes containing rare amino acids selenium (Se) that has been recognized as a key component of the active site. Four distinct groups of GPx isozymes have been characterized: cellular GPx (GPx1), gastrointestinal GPx (GPx2), plasma GPx (GPx3), and phospholipid hydroperoxide GPx (GPx-4 or PhGPx). This paper is mainly focus on GPx1 [2].

Physical Properties and Structure of GPx1

GPx1 is an enzyme containing four identical subunits and each subunit has one active site of selenocysteine residue [3]. The total molecular weight of GPx1 ranges from 75 kDa to 100 kDa. It differs not only from species to species, but also from tissue to tissue, 76±1 kDa for rat liver, 84±1 kDa for bovine erythrocyte, and 95±3 kDa for human erythrocyte [4]. GPx1 is present at high level in kidneys, erythrocytes and livers. The enzyme has been purified from many mammalian. Some important molecular and functional parameters of seleno GPx from bovine red blood cells, such as monomer radius, molecular symmetry, isoelectric point and temperature optimum are shown in Table 1 [5].

Table 1. Molecular and Functional Parameters of Seleno GSH Peroxidase from Bovine Red Blood Cells [5].

Molecular weight	M = 84,000 (tetramer)
Subunits	M = 21,000 (identical)
Monomer radius	R = 19 Å
Molecular symmetry	222
Isoelectric point	pH (I) = 5.8
pH optimum	pH = 8.8
Temperature optimum	T = 42°C
Prosthetic groups	1 selenocysteine/monomer
Crystal space group	C2, monoclinic
Unit cell constants	a = 90.4 Å; b = 109.5 Å, c = 58.2 Å, β = 99°
Subunits per asymmetric unit	dimer, M = 42,000
Packing density	V_m = 3.4 Å^3/dalton

Usually, GPx1 is mapped to chromosome 3p21.3 [6] and consist of about 180± 30 amino acids and the selenocysteine residue is located about 47 residue from the N- terminal [3]. The three-dimensional structure of bovine erythrocyte GPx at 2.8 Å resolution has been determined [7]. Subunits with a radius of 18.7A are nearly spherical. GPx1 is a tetrameric protein composed of two dimers and is almost flat with dimensions of 90.4×109.5×58.6A. The four active sites are located on the surface and Se atoms are found in each active site. The selenium atoms in each dimmer are 21A apart [4]. The large distances of the selenium atoms in tetramer allow the formation of intramolecular diselenide bridges during catalysis, which plays an important role in enzyme function. The peptide chain of the subunit can be traced know a better understanding of the formation and arrangement of active center [8]. Each active site consists of four protein loops; the amino acid sequences 145 to148, 63 to 69 that are also part of the active site of the adjacent subunit. At the positions 30 to 40, contains the active site of selenocysteine, depending on the functional state of the enzyme. The peptide chain and the location of selenium in GPx1 are shown in Figure 1.

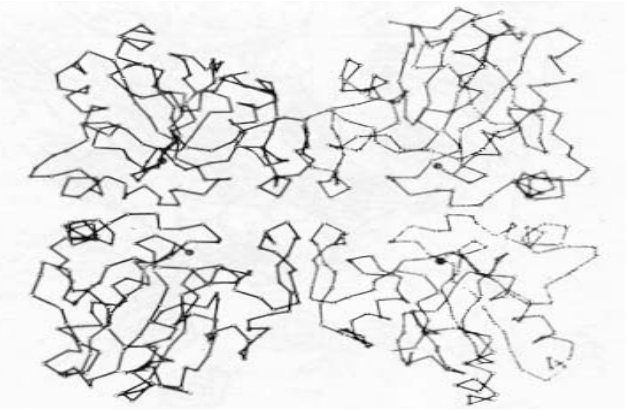

Figure1: Stereo diagram of CA backbone of bovine GSH peroxidase tetramer. Open circles indicate the position of α-carbon atoms. Filled circles show the position of selenium atom [8]

Chemistry of GPx1

The trace element selenium has been demonstrated as a functional group. The enzyme's primary structure includes an analog of cysteine, selenocysteine, with Se replacing S.

Se is important for the enzymatic activity of GPx1. Se can release under the condition of pH < 2, or > 8, or exposure of either pronase or $NaBH_4$. Release of Se from the enzyme or presumably alkylate Se on enzyme can irreversibly lose the function of the GPx1 activity. X-ray photoelectron spectroscopic studies show that the protein-bound selenium undergoes reversible substrate-induced redox-selenol derivatives (R-SeH) in reduced form and a seleninyl (R-SeO-OH) or selenenyl (R-Se-OH) in oxidized form [7].

Animal having Se-deficient diets show rapid decrease in tissue GPx1 level and can show rapid increase in GPx1 activity through Se re-supplementation. It has been found that a single large oral dose of selenite or selenomethionine given to Se-deficient rats resulted in significant increases in kidney, liver and stomach GPx1 activity after 48 hours [4]. Iodoacetate selectively inhibits the enzyme by reacting with the selenocysteine residue in GPx1. The inhibition can be reversed by addition of an amount of hydroperoxides stoichiometric with the selenocysteine residues [4,6].

Kinetic mechanism of GPx1

It has been demonstrated that a substrate-dependent reversible redox change of the selenocysteine residue is the basic catalytic process in the function of GPx1. Substrate-GPx1 readily reoxidizes spontaneously and generally extreme precautions are required to preserve selenocysteine-containing enzymes in physiological state. The kinetics of GPx1 studies under steady conditions shows the ping-pong mechanism. The first step of the catalytic cycle of GPx1 can be described as the oxidation of an ionized selenol by a hydroperoxides to produce a selenenic acid derivation (reaction 1). Evidence shows that a simple transition complex rather than a specific enzyme-substrate complex mediates the reaction in reaction 1 because a wide range of hydroperoxides susceptible to this reduction [9]. The selenol can be regenerated following by two consecutive reactions involving the selenenic acid residue and GSH. Intermediate of a selenosulfide, E-CysSeOH is formed between the enzyme and GSH (reaction 2,3) [6]. The mechanism for these reactions is shown in Figure 2.

$$\text{E-CysSe}^- + \text{H}^+ + \text{ROOH} \xrightarrow{k_1} \text{E-CysSeOH} + \text{ROH} \qquad (1)$$

$$\text{E-CysSeOH} + \text{GSH} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{E-CysSe-SG} + \text{H}_2\text{O} \qquad (2)$$

$$\text{E-CysSe-SG} + \text{GSH} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \text{E-CysSe}^- + \text{GSSH} + \text{H}^+ \qquad (3)$$

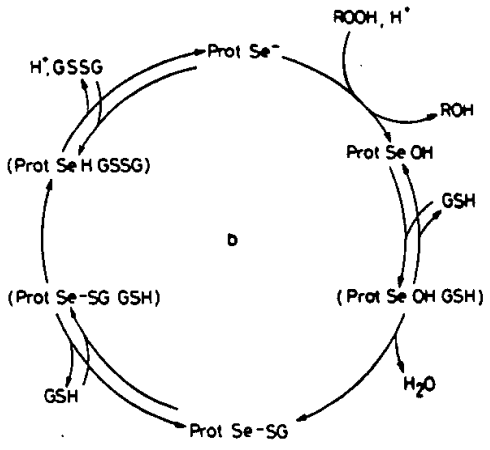

Figure 2. The ping-pong mechanism for GPx[12].

The steady-state treatment of reaction 1-3 yields an initial velocity exactly describing the kinetic pattern of hydroperoxidase and GPx (equation 4). When defining $Ø_1$ and $Ø_2$ as the kinetic coefficients: $Ø_1=1/k_5$, $Ø_2=1/k_{+6} + 1/k_{+7}$[9,10], the kinetic of these reactions fit the Dalziel equation (equation 5). [E] is the enzyme concentration and V is velocity. The velocity equation exactly can be described as the equation 4.

$$-\frac{d[\mathrm{ROOH}]}{dt} = v = [E_o]\left\{\frac{1}{k_{+1}[\mathrm{ROOH}]} + \frac{1}{k_{+2}[\mathrm{GSH}]} + \frac{1}{k_{+3}[\mathrm{GSH}]}\right\}^{-1} \qquad (4)$$

$$[E] / V = Ø_1 /[ROOH] + Ø_2 /[GSH] \qquad (5)$$

The time course of GSH oxidation can be obtained by measuring the NADPH oxidation in a reaction mixture containing the peroxidic substrate and the GSH regenerating system. The absorbance is determined until all hyperoxides consumed. The initial concentrations of hydroperoxides as well as the concentration of the reduced product (ROH) are calculated from NADPH absorbance. The concentration of GSH is constant by GSSG reductase and NADPH. Because [GSH] is a constant and [E] and [ROOH] are known, it is possible to measure $Ø_1$ and $Ø_2$. Then rate constants for the interaction of GPx1 with peroxide substrate could be calculated. Usually k_{+2} and k_{+3} cannot be differentiated [10].

For H_2O_2, the k_5 values of the tetrameric bovine enzyme range between 0.6 and 1.8×10^8 M^{-1} sec^{-1} depending on the incubation medium. Rate constants are also observed with organic hydroperoxides such as ethyl hydroperoxide ($k_1= 3 \times 10^7$ M^{-1} sec^{-1}), cumene hydroperoxides ($k_1= 1.3 \times 10^7$ $M^{-1}sec^{-1}$), and ter-bytyl hydroperoxide ($k_1= 0.75 \times 10^7$ M^{-1} sec^{-1})[8].

The apparent second- order rate constant for the interaction between the hydroperoxides and the GPx1 and PhGPx are showed in table1. It shows that k_{+1} value for H_2O_2 is higher in reaction

with GPx1 than with PhGPx [5]. However, PhGPx can directly and rapidly reduce phospholipid hydroperoxides that are not substrates for GPx1 [10].

Table 2. Apparent second order rate constant for the reaction between GPx and PhGPx using hydroperoxidic substrate. Adapt from [11]

Substrate	GPx k_1 (M^{-1} s^{-1})	PhGPx k_1 (M^{-1} s^{-1})
Hydrogen peroxide	5×10^7	3.2×10^6
Cumene hydroperoxide	2.1×10^7	2.2×10^6
Linoleic acid hydroperoxide	3.1×10^7	3.9×10^7
Phosphatidyl choline hydroperoxides	———	1.2×10^7

The rate constants are calculated from the linear plots of integrated rate equations as described above. The GSH is 3 mM and the hydroperoxides 60nm.

Reaction of GPx1

Superoxide, a powerful oxidizing agent, is produced in the oxidative stress, and then rapidly reduced to H_2O_2 by SOD (reaction 6). The rate constant for this reaction depends on pH. At pH 7.0, $k = 5 \times 10^9\ M^{-1}s^{-1}$ [12].

H_2O_2 can be converted to H_2O by the function of GPx. In fact, GPX has been shown not only to react with H_2O_2 (reaction 7), but also has the function catalyzes degradation of organic hydroperoxides by reduction two glutathione molecules oxidizing to a disulfide (reaction 8)[12-13]. The rate constant of GPx with H_2O_2 is determined about 2×10^8 or $5 \times 10^7\ M^{-1}\ s^{-1}$, after taking subunit activity is taken into consideration [14]. The rate constant of GPx with organic hydroperoxides ranges from $0.6 \times 10^7 \sim 3 \times 10^7\ M^{-1}\ s^{-1}$, depending on different kind of hydroperoxides. Regeneration of reduced glutathione requires NADPH produced in the Pentose Phosphate Pathway. Glutathione reductase catalyzes the reaction of NADPH reducing GSSG to form 2 GSH (reaction 9).

$2O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2$ $k_6 = 5 \times 10^9\ M^{-1}\ s^{-1}$ [12] (6)

$2\ GSH + H_2O_2 \xrightarrow{GPx} GSSG + 2H_2O$ $k_7 = 5 \times 10^7\ M^{-1}\ s^{-1}$ [14] (7)

$2\ GSH + ROOH \xrightarrow{GPx} GSSG + ROH + H_2O$ $k_8 = 0.6 \times 10^7 \sim 3 \times 10^7\ M^{-1}\ s^{-1}$ [8] (8)

$GSSG + NADPH + H^+ \xrightarrow{GR} 2\ GSH + NADP^+$ (9)

Summary:

GPx1 is a kind of Se-dependent enzyme. It is an antioxidant that involves in defence against peroxidarion and prevents the breakdown of hydroperoxides from formation of new radical. GPx1 structure has been well characterized, the selenocysteine residue in this protein is the basic catalytic process in the function of GPx. The kinetics of GPx studies under steady conditions shows the Ping Pong mechanism.

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