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Understanding mammalian glutathione peroxidase 7 in the light of its homologs

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ABSTRACT

The glutathione peroxidase homologs (GPxs) efficiently reduce hydroperoxides using electrons from glutathione (GSH), thioredoxin (Trx), or protein disulfide isomerase (PDI). Trx is preferentially used by the GPxs of the majority of bacteria, invertebrates, plants, and fungi. GSH or PDI, instead, is preferentially used by vertebrate GPxs that operate by Sec or Cys catalysis, respectively. Mammalian GPx7 and GPx8 are unique homologs that contain a peroxidatic Cys (C_P). Being reduced by PDI and located within the endoplasmic reticulum (ER), these enzymes have been involved in oxidative protein folding. Kinetic analysis indicates that oxidation of PDI by recombinant GPx7 occurs at a much faster rate than that of GSH. Nonetheless, activity measurement suggests that, at physiological concentrations, a competition between these two substrates takes place, with the rate of PDI oxidation by GPx7 controlled by the concentration of GSH, whereas the GSSG produced in the competing reaction contributes to the ER redox buffer. A mechanism has been proposed for GPx7 involving two Cys residues, in which an intramolecular disulfide of the C_P is formed with an alleged resolving Cys (C_R) located in the strongly conserved FPCNQ motif (C86 in humans), a noncanonical position in GPxs. Kinetic measurements and comparison with the other thiol peroxidases containing a functional C_R suggest that a resolving function of C86 in the catalytic cycle is very unlikely. We propose that GPx7 is catalytically active as a 1-Cys-GPx, in which C_P both reduces H_2O_2 and oxidizes PDI, and that the C_P-C86 disulfide has instead the role of stabilizing the oxidized peroxidase in the absence of the reducing substrate.

Introduction

The glutathione peroxidase homologs (GPxs)[1] are tetrameric or monomeric enzymes sharing an extended thioredoxin (Trx) fold (Fig. 1), a highly conserved catalytic center, and either a Sec or a Cys residue as the redox-active moiety (SecGPx or CysGPx, respectively) (Fig. 2).

GPxs catalyze the reduction of a hydroperoxide (ROOH) by two thiol groups (RSH) according to the general equation

$$ROOH + 2RSH \rightarrow ROH + H_2O + RSSR, \quad (1)$$

where ROOH may be H_2O_2, peroxynitrite, or an aliphatic hydroperoxide (see below), and RSH, depending on the differing GPx specificities, may be glutathione (GSH) or protein thiols, typically those containing a CXXC motif as in Trx, protein disulfide isomerase (PDI), glutaredoxin, or vicinal Cys residues, as in the sperm mitochondrial capsule cysteine-rich protein.

As several comprehensive reviews on GPxs are available [1–4], here we will briefly provide some general mechanistic and structural aspects, as a background for understanding the newly described and still mysterious GPx7, which is the focus of this review.

GPxs use an "enzyme-substitution" mechanism in which the hydroperoxide substrate (ROOH) oxidizes the reduced enzyme (E_{red}) to the oxidized species (E_{ox}) in a nucleophilic substitution reaction. The catalyst is then regenerated by the reducing

Abbreviations: C_P, peroxidatic Cys; C_R, resolving Cys; CysGPx, glutathione peroxidase containing cysteine as the redox-active moiety; 1-Cys-GPx, glutathione peroxidase using the peroxidatic Cys only for catalysis; 2-Cys-GPx, glutathione peroxidase using the peroxidatic Cys and the resolving Cys for catalysis; *Br*GPx, *Brassica rapa* glutathione peroxidase; *Dm*GPx, *Drosophila melanogaster* glutathione peroxidase; ER, endoplasmic reticulum; E_{red}, reduced enzyme; E_{ox}, oxidized enzyme; GPxs, glutathione peroxidase homologs; *h*PDI, human protein disulfide isomerase; PLOOH, phospholipid hydroperoxides; ROOH, hydroperoxide; PDI, protein disulfide isomerase; *Pf*GPx, *Plasmodium falciparum* glutathione peroxidase; Prxs, peroxiredoxin homologs; RSH, thiol group; RSSR, oxidized thiol; SecGPx, glutathione peroxidase containing selenocysteine as the redox-active moiety; –SOH, *S*-hydroxycysteine; –SeOH, Se-hydroxyselenocysteine; *Tb*GPx, *Trypanosoma brucei* glutathione peroxidase; Trx, thioredoxin.

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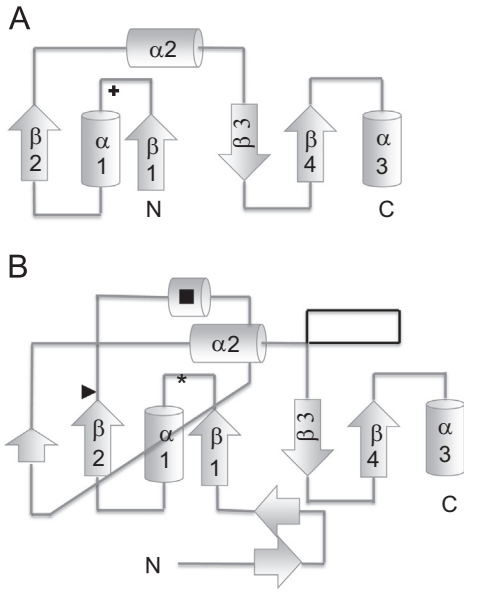

Fig. 1. The (A) Trx fold and (B) GPx fold architecture. In (A) the cross indicates the position of the N-terminal C of the CGPC redox motif of Trx. In (B) the additional sheets and helices of the GPx fold are shown. Here, the asterisk indicates the position of the catalytic moiety (Sec in SecGPx or Cys in CysGPx). Note that this corresponds to the N-terminal C of the CGPC redox motif of Trx. The black triangle at the C-terminal end of the β2 sheet indicates the position of the Cys within the conserved FPCNQ motif. The black square inside the additional helix represents the "Cys block," i.e., the amino acid stretch where, in the invertebrate, plant, or fungus 2-Cys-GPx, the C_R residue is located. The black line represents an amino acid stretch composing the tetrameric interface that is deleted in the monomeric members (see also Fig. 2).

equivalents provided by an RSH substrate, which is eventually released in the oxidized form (RSSR):

$$\mathrm{ROOH} + \mathrm{E_{red}} \overset{k_{+1}}{\rightarrow} \mathrm{E_{ox}} + \mathrm{ROH} + \mathrm{H_2O}, \quad (2)$$

$$\mathrm{E_{ox}} + 2\mathrm{RSH} \overset{k'_{+2}}{\rightleftarrows} \mathrm{E_{red}} + \mathrm{RSSR}. \quad (3)$$

k_{+1} and k'_{+2} calculated by steady-state kinetic analysis represent the apparent rate constants for Eqs. (2) and (3), respectively, where k'_{+2} is a cumulative rate constant, accounting for the average of the rate constants of the two independent reducing steps, which cannot be independently calculated: the formation of a first intermediate between E_{ox} and the first thiol, also called the semireduced enzyme intermediate (Eq. (3′)), and the release of the disulfide when E_{red} is regenerated (Eq. (3″)):

$$\mathrm{E_{ox}} + \mathrm{RSH} \rightleftarrows \mathrm{E\text{–}SR}, \quad (3')$$

$$\mathrm{E\text{–}SR} + \mathrm{RSH} \rightleftarrows \mathrm{E_{red}} + \mathrm{RSSR}. \quad (3'')$$

Specificity for the hydroperoxide substrate differs considerably among the various homologs. With few exceptions, the tetrameric GPxs react with H_2O_2 or small fatty acid or other organic hydroperoxide, whereas the monomeric GPxs also accept much larger substrates, such as phospholipid hydroperoxides (PLOOH). Reactivity with PLOOH seemingly is allowed by the absence of the loop containing the tetrameric interface that hinders the access of large molecules to the flat depression surrounding the catalytic center containing the Se/S atom [5]. No specific constraints have been identified for the interaction of H_2O_2 with the active site of the individual GPxs. However, in the monomeric SecGPx GPx4, the presence of a cationic area proximal to the redox center [1] has the apparent role of directing, via polar interaction with the phosphate group of the phospholipids, the optimal interaction of the hydroperoxy fatty acid with the redox center. Yet, there are two documented exceptions to this pattern of interaction: (i) vertebrate GPx3, a tetrameric SecGPx for which reactivity with PLOOH has been reported [6], and (ii) the GPx of *Plasmodium falciparum* (*Pf*GPx), a monomeric peroxidase that apparently does not react with PLOOH [7]. Notably, in GPx3, the tetrameric interface is restricted to a short loop, a feature that most likely limits specificity through steric hindrance (Fig. 2).

Although the term "peroxidase," which unambiguously defines a catalyst for reducing an ROOH, is applicable to all the GPx homologs, *glutathione* peroxidase as a functional definition of the enzyme family is misleading, because GSH is not the specific reducing substrate of most GPxs. As a matter of fact, these homologs were named after the first discovered glutathione peroxidase, currently known as GPx1 [8], on the basis of structural and conformational homology, retaining the name GPx while disregarding the specificity for the reducing substrate. Therefore, "GPx" today refers to enzymes that share structural homology and catalyze the transfer of reducing equivalents from various thiols to ROOH.

A classification based on the kinetic data of the reducing part of the catalytic cycle can be proposed. The value of k'_{+2}, combined with structural and conformational similarity, substantiates three subgroups of GPx homologs: (i) the "real" glutathione peroxidases, (ii) the thioredoxin peroxidases, and (iii) the protein disulfide isomerase peroxidases. The specific features of each subgroup are briefly summarized before we propose the new category of PDI peroxidases, to which GPx7 belongs.

The "real" glutathione peroxidases

The "real" GPxs are tetrameric or monomeric peroxidases using a catalytic Sec and GSH as reducing substrate (GPx1, GPx2, GPx3, GPx4, and human GPx6). Kinetic analyses, available for GPx1, GPx3, and GPx4 (Table 1), indicate that these enzymes, after a very fast oxidation by H_2O_2, are rapidly reduced by GSH. Although GPx2 and human GPx6 have not been kinetically characterized, the profound similarity to GPx1 and GPx3 strongly suggests that these enzymes also react rapidly with both H_2O_2 and GSH. The prototype of this subgroup is the tetrameric GPx1. As shown in Table 1, human GPx1 is oxidized by H_2O_2 (Eq. (2)) with a k_{+1} value of $4.1 \times 10^7\ \mathrm{M^{-1}\ s^{-1}}$, which is rapid enough to prevent the accumulation of an enzyme substrate complex, a kinetic feature that produces non-saturation kinetics, which do not comply with the Michaelis–Menten theorem. The reducing step by GSH has a k'_{+2} value of $2.3 \times 10^5\ \mathrm{M^{-1}\ s^{-1}}$, which, although rate limiting for the catalytic cycle (Eq. (3)), is very fast indeed (Table 1).

Bovine GPx1 was the first crystallized GPx and its sequence was first tentatively deduced from the electron density distribution [9]. With the sequence established by Edman degradation [10], a revised version of the structure was published 3 years later [11]. Within the crystal, the refined electron-density map of the oxidized enzyme indicated that selenocysteine was oxidized to a seleninic acid derivative. This led to the hypothesis that the functional form of GPx1 oxidized by the hydroperoxide (i.e., E_{ox} in Eq. (2)) is a Se-hydroxyselenocysteine (i.e., the selenenic acid derivative of the catalytic Sec, –SeOH). However, it is still a matter of debate whether –SeOH is really the stable oxidized form in SecGPxs. Indeed, owing to the extreme reactivity of this species, this is extremely unlikely. If GSH is available, –SeOH reacts, forming a mixed selenodisulfide that has been verified by MS analysis in GPx4 [12]. In the absence of GSH, however, the presumed –SeOH form rearranges, forming, by water loss, a species that is 2 amu lower than the reduced form [12], the structure of which has not yet been resolved.

Although kinetic studies supported the assumption that SecGPxs generally use GSH as a reducing substrate (Table 1), only