

Structural and Functional analysis of glutathione peroxidase from *Ricinus communis* L. – a computational approach.

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Abstract- Oxidative stress in plants causes the induction of several enzymes, including superoxide dismutase (EC 1.15.1.1), ascorbate peroxidase (EC 1.11.1.11) and glutathione reductase (EC 1.6.4.2). The first two are responsible for converting superoxide to H₂O₂ and its subsequent reduction to H₂O, and the third is involved in recycling of ascorbate. Glutathione peroxidases (GPxs, EC 1.11.1.9) are a family of key enzymes involved in scavenging oxyradicals in animals. Only recently, indications for the existence of this enzyme in plants were reported. Genes with significant sequence homology to one member of the animal GPX family, namely phospholipid hydroperoxide glutathione peroxidase (PHGPx), were isolated from several plants. In this paper we report the homology modelling of the glutathione peroxidase protein from *Ricinus communis* L. and its interactions with its two substrates hydrogen peroxide and glutathione. Specific sites of interaction were identified and ligand binding pockets were also screened.

Keywords: *Ricinus communis* L. Delaunay triangulation, glutathione peroxidase, oxidative stress, accessible surface area, pocket identification

Introduction

Reactive oxygen species (ROS) are generated through an incomplete reduction of oxygen molecules during mitochondrial respiration and/or cytosolic metabolism. Exposure to exogenous stimuli such as radiation and redox cycling drugs might be an alternative pathway of ROS production. ROS perform physiological roles relevant to cell signaling and redox-status control [1,2], while unbalanced generation of these species induces detrimental oxidation of macromolecules including DNA, proteins, and lipids. To minimize ROS-derived damage, aerobic organisms have evolved a series of multi-layered enzymatic and non-enzymatic defense systems [3]. Distinct enzymatic activities such as catalase, glutathione peroxidase (GPx), and peroxiredoxin (PRx; also called thioredoxin peroxidase) have been well characterized from numerous taxa, as the major antioxidant defense mechanism. Selenium-containing GPx proteins reduce H₂O₂ and organic hydroperoxides by employing glutathione (GSH) as an electron donor. A total of eight GPx families have been described in mammals on the basis of primary structure, specific substrate accessibility, and spatial expression [4,5]. These homotetrameric isoenzymes conserve structural/ biochemical properties, however, a number of enzymes that have been classified into GPx4 (phospholipid hydroperoxide GPx; PHGPx) may function in monomeric forms and exhibit unique substrate availability. The enzymes can interfere directly with hydroperoxidized phospholipids in biomembranes. Proteins belonging to the other GPx families display substrate preference toward H₂O₂ and protect against lipid peroxidation via a concerted operation with phospholipase [6].

PHGPx is the basis of a principal defense system that intimately participates in the repair of disrupted biomembranes [7]. The vertebrate-specific GPx7 and GPx8 also lack the oligomerization loop, although their unique enzymatic properties are less understood [5]. Multiple isoenzymes showing primary structure similar to those of the mammalian PHGPxs have been described in plants, along with their respective subcellular expression profiles [8,9]. Plant enzymes possess a Cys residue instead of a selenocysteine (Sec) at the catalytic site, and prefer thioredoxin (Trx) as the electron source [9-11]. A pair of PHGPx-like proteins that effectively reduce the peroxides by adapting the Trx system has also been isolated from insect, yeast, and protozoa [12-15]. Interestingly, the green alga *Chlamydomonas reinhardtii* was likely to express both GSH-dependent (CrGPx1 and CrGPx2) and Trx-dependent (CrGPx3-5) GPxs [16]. These observations have created a controversy regarding the classification of PHGPx-like proteins [8,9]. Conversely, a novel functional class of 'Trx GPx-like peroxidase (TGPx)' has been proposed to clarify the unique GPx group sharing a common evolutionary origin with the GSH-dependent GPxs [5]. The molecular basis for the differential preference has also been investigated and appeared to involve a 'resolving Cys' within the α 2 helix of the Trx-dependent GPxs [5,18,19]. In this work we structurally analyze the *Ricinus communis* L., glutathione peroxidase, isolated under conditions of lead accumulation.